

Comparison of Two Methods of Measuring Body Composition in HIV-infected Children (9-36 Months) at Initiation of Highly Active Antiretroviral Therapy (HAART)

Carey Anne Haupt

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DECLARATION

I, Carey Anne Haupt declare that this dissertation is my own work. It is being submitted for the degree of Master of Science in Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University

Carey Haupt

.....24..... day of February..... Month 2014

DEDICATION

To Colin, my loving Husband and two children.

ABSTRACT

Background

The relationship between HIV and body composition is not well documented. No description of Fat Mass (FM), Fat Free Mass (FFM) or body fat percentage (BF%) has been reported in children infected with HIV (9-36 months), before initiation of antiretroviral treatment (ART). This is mainly due to the lack of a validated in-field method for measuring body composition in HIV infected children. Regression equations to estimate FM or BF% have not been developed for young South African children. Guidelines however recommend that equations developed and validated elsewhere be used in this population. For the recommended guidelines to be used with confidence in South African children they need to be validated.

Objectives

To describe the body composition of 36 treatment naïve HIV-infected children (9-36 months) and to compare the BF% and FM (g) results of four regression equations to those obtained from Dual Energy X-ray's (DXA).

Methods

This is a retrospective, observational, cross sectional study. It includes descriptive data on body composition of HIV infected children and comparative data between published regression equations and DXA results. The 37 participants were enrolled from Harriet Shezi Children's Clinic and all were aged 9-36 months, had parental consent for the study and had confirmed HIV infection but were ART naïve. Anthropometric measurements and DXA scan were done within a 10 day window period. Secondary analysis of FM and BF%

estimates from DXA scans and four regression equations were tested for agreement using Bland-Altman method.

Results

The DXA results (with head) showed a median BF% 25% (IQR22:32) and FM 1952g (IQR1594:2847) with no significant difference between genders. The fat patterning showed most of the FM in the legs 810g (IQR560:1220), then the trunk 689g (IQR520:930) and arms 13g (1.2:42.1). The trunk had the highest FFM 2700g (IQR2030:2960) of the three regions.

The calculated BF% from the equations were: Slaughter 10% (IQR9:13) and Shaikh & Mahalanabis 19% (IQR18:20). The FM (g) calculated from the equations were: Dezenberg -1823g (IQR-2450:-1160) and Goran 296g (IQR-83:1116). The results of the Bland-Altman analysis showed a high mean biases with wide limits of agreement between DXA and all four of the equations.

Conclusion

This is the first study to describe body composition in HIV-infected South African children (9-36 month) using DXA. The study has shown the importance in validating a regression equation before using it in a new study population, as the agreement between all 4 regression equations and DXA was very poor. The need for further studies in body composition and HIV has been highlighted. Further topics to be studied should include: the use of skinfold sites and girth measurements as in field monitoring of fat patterning, the development of South African specific regression equations for use in HIV infected children, the study of body composition using DXA in healthy and HIV infected children and the role of body composition with regards to absorption and metabolism of ART.

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1 BACKGROUND

1.1 Introduction

The nutritional status of children infected with HIV forms part of the assessment for eligibility for antiretroviral treatment (ART) (1). It is also used as part of the screening of HIV-infected children for opportunistic infections such as TB and to monitor response to ART (1). A variety of anthropometric parameters are used to measure nutritional status these include weight, height, mid upper arm circumference (MUAC), waist circumference, and triceps and subscapular skinfold thicknesses (2). Since children are still growing most anthropometric parameters are compared to age and sex specific standardized population reference ranges such as growth charts or Z-scores. The most commonly used charts for children are the WHO growth standards and reference charts.

The anthropometric parameters used to assess the nutritional status of children have led to a large number of terms that are used to describe growth abnormalities. While these terms are sometimes used incorrectly and interchangeably in the literature, they have specific meanings according to the WHO classification of nutritional status (3). The terms used here are in keeping with those of the WHO:

- Weight-for-age (WAZ) < -2 Z scores as underweight

- Weight-for-height (WHZ) < -2 Z scores as wasted
- Height-for-age (HAZ) < -2 Z scores as stunted
- Body Mass Index (BMI) < -2 Z scored as wasted
- Mid upper arm circumference (MUAC) < 115mm for severe acute malnutrition (SAM)

While anthropometric measurements are certainly useful, the assessment of nutritional status can be taken a step further by measuring body composition. Body composition is the study of the different components of the body: fat mass (FM), fat free mass (FFM) and percentage body fat (BF%). For example FM and BF% have been associated with clinical outcomes and are therefore of clinical importance (4). Increased FM and/or BF% is associated with increased risk of insulin resistance, abnormal blood lipids and diabetes mellitus in adults infected with HIV (5). While a loss of FFM has been associated with a decrease in quality of life (6).

1.1.1 HIV and nutritional status of children

Since HIV has a dramatic impact on the growth and nutritional status of children, with wasting being one of the first discernible clinical signs, the assessment of body composition in HIV-infected children could be of significant clinical and/or prognostic value. One of the first terms to describe HIV was slim disease (7). The term “slims” highlighted the relationship between HIV and nutritional status including weight loss in adults and growth failure in children. “Growth failure” or “failure to thrive” are terms that

indicate that the weight/height or rate of weight/height gain is significantly lower than children of a similar age and gender. Growth failure in children, including stunting, weight loss or being underweight, is associated with increased mortality and significant morbidity (8). The effects of growth faltering have been shown to extend into adult life, with poor nutritional status in children having a sustained detrimental effect on development and ability to function as an adult later in life (9).

The profound negative impact of HIV on children's growth has been described since 1993 (8, 10, 11). Longitudinal studies conducted in the United States, Europe and Africa have shown that growth failure is one of the first markers of HIV infection, affecting both weight-for-age and length-for-age. Growth failure is exhibited in one third of HIV-infected children (6, 12-16). Signs of growth failure have been reported from as early as 3 months of age (10, 14). HIV-infected children with growth failure tend to have higher viral loads (6). Even with the initiation of HAART, wasting and weight loss can still take place (17). The mechanisms by which HIV affected growth are not yet fully understood. It is hypothesized that HIV may compromise nutritional intake, alter the absorption and metabolism of nutrients, stimulate excessive cytokine production, and increase the incidence of secondary opportunistic infections, thus increasing energy requirements (12, 17, 18). These mechanisms may act individually or in combination to result in growth failure. Although growth failure and HIV has been extensively researched there are only a few studies that have focused on HIV and body composition.

1.1.2 Body Mass Index

Body Mass Index is a fairly easily obtained and commonly used proxy measure of body fat in adults and is used to determine if an adult is underweight, overweight or obese. It does not measure FM directly and has significant limitations in children. BMI is calculated as weight (kg) divided by height squared (m^2). Adults are classified according to BMI as underweight ($BMI (kg/m^2) < 19$), normal ($BMI (kg/m^2) \geq 19$ and < 25), overweight ($BMI (kg/m^2) \geq 25$ and < 30) or obese ($BMI (kg/m^2) \geq 30$) with high BMI associated with significant obesity related co-morbidities (19, 20). While BMI actually measures excess weight which can either be FM or FFM (21), BMI is widely accepted as an appropriate measure to screen adults for obesity and related health risks (22).

In older (3-18 years) children BMI has also been shown to have a fairly strong correlation with adiposity (19). However normal BMI ranges change considerably with age (23) and these shifts are sex dependent. In 2000 Cole et al. (23) published proposed international age and sex specific cut offs for BMI for children aged 2-18 years that were intended to correspond with adult BMI of 25 and 30 (23). The aim of the study was to identify an acceptable definition for childhood obesity and overweight however the authors acknowledged that the obesity cut offs values were fairly imprecise and likely to be less accurate than the overweight values (23). Subsequently, WHO published their age and sex specific BMI ranges for children in 2007 which are now commonly used in children (24).

While BMI has been accepted as part of routine weight assessment it is important to understand its limitations in predicting FM in children. Firstly, both height and level of

sexual maturation influence the relationship between FM and BMI (22). Secondly the association between BMI and amount of body fat is also partly dependent on how much fat the individual child has (22). For example while an obese child's BMI is generally a good indicator of excess body fat, in an overweight child the elevated BMI could be due to either excess FM or FFM or both (22). In a thin child, a low BMI is often partly explained by a loss of FFM. Furthermore, in younger children BMI tends to overestimate FM and obesity (20). In very young children BMI has only a weak association with FM (20).

The association between BMI and BF% (measured by DXA) in healthy children was explored in a New Zealand study of 661 Caucasian children (19). It was found that at three years of age a BMI (kg/m^2) of 25 corresponded with a BF% of 20% (95% CI: 19 ; 21) for males and 21% (95% CI: 19 ; 21) for females. As for a BMI of 30, the corresponding BF% was 26% (95% CI: 25 ; 29) for males and 26% (95% CI: 25 ; 27) for females. However, generalisation of these results to other populations might be inappropriate as the association between BF% and BMI might differ in other populations (19). A study conducted in 2000 African American and Caucasian children, aged 4-11 years that examined obesity (FM measured by DXA) and biological risk factors (lipid profile and blood pressure) found two cut-off points. Firstly, children with a BF% $\geq 33\%$ were approximately 15 times more likely to have high risk lipid and blood pressure profiles than a normal risk profile. However, no confidence intervals provided as this was based on analysis of Receiver Operator Curves. Secondly children with a BF% of $<20\%$ were 0,16 times less likely to have a high risk lipid and blood pressure profile than a normal risk profile (20).

While obesity, excess body fat and disorders of body fat distribution are becoming more prevalent in the post-ART era, individuals infected with HIV are generally more likely to be wasted or malnourished, particularly prior to ART initiation. A published definition of under-nutrition based on FM in children aged younger than 18 years of age determined that appropriate cut-offs of BF% were <5% for boys and <12% for girls (25). With regards to the WHO BMI Z-scores, children with a BMI <-2 are considered wasted. The actual values for the -2 Z-score are given in Table 1.1. Thus these parameters can be used when using BMI or BF% to determine a child's risk profile or weight classification.

Taylor's (19) classifications and Higgins' (20) cutoffs were designed for children aged three and four to eleven, respectively. This limits the acceptability of using these classification and cut offs to interpret DXA BF% in children under the age of three. However there are no recommended BF% guidelines for healthy, overweight or obesity for children under the age of three. Thus the use of Taylor's classification and Higgins cut offs in children under three would be done with an understanding of the limitations of using classifications in a younger age group. A further limitation of Higgins (20) cut off is that his cut off point for reduced risk for biological risk factors for BF% are < 20% for males and < 21% for females, but does not have a lower cut off for under nourished. Thus it is difficult to interpret these results as a child with a BF% of < 5% for males and <12% for females would be classified by Higgins as low risk but undernourished according Reilly's (25) cutoffs to and therefore at high risk of mortality.

Table 1.1 is a summary and comparison of the classifications and cutoffs for BMI and BF% described above. It is evident in the table that the classifications and cut offs are not in agreement which inhibits the interpretation of the table. In an attempt to condense and make the recommendations comparable the table separates out the recommended cutoffs into underweight, normal (low biological risk), overweight and obesity.

Table 1.1: Summary of BMI and BF% and risk profiles for children

Age(1)	BMI		BF%		Comments
	Male	Female	Male	Female	
Underweight					
< 18 years (25)	-	-	<5%	<12%	Definition of under nutrition
Normal (low risk) ranges					
9 months (26)	14,7-20,3	14,1-20,1	-	-	Normal range for age and sex age (<-2 ; <2)
3 years (26)	13,4-18,4	13,1-18,4			Normal range for age and sex age (<-2 ; <2)
4 to 11 years (20)	-	-	<20%	<21%	Decreased lipid and blood pressure risk profile
Overweight ranges					
2 to 3 years (23)	18,1	17,8	-	-	Classified as overweight
3 to 18 years (19)	25	25	20% CI (19 ; 21)	21% CI (19 ; 21)	Classified as overweight
Obese ranges					
2 to 3 years (23)	19,8	19,6	-	-	Classified as obese
3 to 18 years (19)	30	30	26% CI (25 ; 29)	26% CI (25 ; 27)	Classified as obese
4 to 11 years (20)	-	-	33%	33%	15 times more likely to have high risk cardiovascular risk profile

Given the limitations of BMI in non-obese children, particularly younger children, the association between BMI and BF% in HIV-infected children needs to be assessed in order to aid interpretation of BMI results in this population.

1.1.3 HIV and changes in body composition

There are indications that body composition could be potentially useful in monitoring treatment and evaluating clinical outcomes in HIV-infected adults and children. A decrease in FFM has been associated with disease severity, higher HIV viral load, mortality, decrease in muscle protein mass, decrease in quality of life, reduced immune function, reduced tolerance of ART therapy and an increased cost of medical care (6, 17). Body composition may alter the pharmacokinetics of various drug, resulting in altered concentration of drug at the site of action, altered efficacy or increased risk of adverse drug reactions (27). More specifically to children infected with HIV, is that malnutrition can interfere with the absorption of HAART (28). In addition with the introduction of HAART, there has been an increase in the number of people who are HIV-infected with elevated FM and/or BF%. It is currently not known if obesity has a protective or harmful effect on HIV disease progression (5).

A literature search using PubMed and Google scholar was conducted with the following search terms: body composition, FM, FFM, BF%, preschool, toddler, infant, HIV/AIDS, lipodystrophy, DXA and skinfold thickness. The literature search identified seven studies of body composition conducted in HIV-infected children (Table 1.2). These studies examined the relationship between HIV, FM and FFM in children using a variety of body

composition techniques. DXA and then skinfold thickness were the most commonly used techniques.

Table 1.2: HIV and body composition studies in children

Author	Study design	Population	Age	ART	Country / City	Method used to measure FM, FFM or BF%	Main findings
Fontana (1999)	Cross-sectional	HIV-infected and uninfected children	Mean age of 6.9 – 7.7 years	No	Milan	Mid upper arm circumference Tricep skinfold and Bioelectric Impedance Analysis (BIA)	A decrease in FFM is common in HIV infected children that have moderate to severe Symptoms of AIDS but not in children with no or mild symptoms
Arpadi (1998)	Cross-sectional study of body composition	HIV-infected and uninfected children stratified by presence of growth failure. Growth failure was defined as 12 month height velocity of the fifth percentile or lower for age.	4-11 years	85% of HIV infected children on ART	USA New York	DXA, tricep skinfold mid-upper-arm-circumference and potassium counting	Boys and girls who had HIV associated growth failure also exhibited decreased FFM while their FM was within the normal range. However boys without growth failure also had decreased FFM whereas girls without growth failure did not, suggesting that body composition may be affected

							differently in boys and girls (8).
Jacobson (2010)	Cross-sectional descriptive study comparing HIV – infected and HIV-uninfected but exposed children	303 HIV-infected and 115 uninfected children	7-16 years	Yes	USA 15 different cities	DXA	HIV-infected children had a lower FM than HIV-uninfected. A possible explanation for the decreased total body fat was the use of Protease Inhibitor (PI) for longer than two years. Also reported in the study was the children's trunk to extremity ratio of 0,83 for males and 0,84 for females (29).
Brambilla (2001)	Cross-sectional study comparing children and infected with HIV with or without lipodystrophy	34 healthy children (matched pairs) 34 HIV-infected children: 6 Lipodystrophy 28 without lipodystrophy	6.5 – 16.9 years	Yes	Italy Milan	DXA Magnetic resonance imaging (MRI)	All of the children that received HAART had a distinct increase in central FM and decreased peripheral FM. Only children with true lipodystrophy had true central obesity. DXA was able in the absence of clinical lipodystrophy to

							detect changes in FM (30).
Chantry (2010)	Longitudinal observational study	97 HIV-infected children starting or changing HAART	1 month to 13 years	71% on ART	Multiple international sites	Skinfolds and circumferences BIA	HIV-positive children who started antiretroviral therapy (ART) or changed regimes had improved growth and FFM (31).
Miller (1993)	Longitudinal observational study	52 HIV-infected and 37 HIV-exposed	<15 months	Yes	USA Boston	Tricep skinfold and mid-upper-arm-circumference MUAC.	HIV-infected children had significantly less (p=0.01) arm FFM than uninfected children although both were still in the normal range (10)
Arpadi (2009)	Longitudinal study comparing healthy and HIV-infected children	64 HIV-infected and 147 HIV-uninfected children	6-16 years	Yes (94% on ARV treatment)	USA New York	DXA scans at baseline and yearly for 2 years	Although the total FM was similar between the groups, the distribution of FM was different. HIV-infected children tended to have less leg fat but more arm and trunk fat than the HIV-uninfected children. This difference increased over time which may

							increase the children's risk of cardiovascular disease (32).
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In all of the studies the majority of children were on ART. The studies describe different relationships that may exist between HIV and body composition. The actual relationship is difficult to extrapolate as the studies are not easy to compare due to compounding factors like: age, gender, study design, type of ART used, length of use and race. Four of the studies found in the literature search by Fontana (33), Arpadi (8), Jacobson (29) and Brambillaa (30) had cross sectional design and compared children infected with HIV to children that were not infected with HIV. Fontana's study compare children that were infected with HIV to children that were not infected with HIV but had been admitted to hospital for minor surgery and who were considered normally nourished. The study found that the children with mild or symptomatic AIDS had less FFM than the children not infected or who did not have any symptoms. Arpadi's 1998 study focused on pre-pubertal children, 85% of the Children infected with HIV were on ART at the time of the study. The children had three types of body composition studies carried out on them: DXA, anthropometry and potassium counting. The main findings of the study was that children who had HIV associated growth failure also had reduced FFM, While FM may have been low, it was still in the normal range. In addition to this it was found that boys who did not have growth failure tended to have less FFM whereas girls did not. This finding suggested that there may be a difference in how HIV affects the body composition in boys and girls. Jacobson (2010) compared children aged 7-19 years who were infected or uninfected with HIV. All of the HIV infected children were on antiretroviral treatment at the time of the study. The body

composition analysis was done by DXA scans. This study found that the HIV infected children had less FM. The authors suggested that this may be due to the fact that the children had been on a protease inhibitor for longer than two years. Another finding was that the children had trunk to extremity ratio of 0.83 for the males and 0.84 for the females. Brambilla's (2001) study focused on the body composition and lipodystrophy using DXA and MRI scans in children aged 6.5-16.9 years. The results showed that DXA was able to detect changes in FM even in the absence of clinical lipodystrophy. Also reported was that children who were on ART had increased central FM and decreased peripheral FM.

The three remaining studies were longitudinal studies. Chantry's (2010) study observed 97 HIV infected children aged 1 month to 13 years with 71% of the children already on ART while the remaining children started ART at the time of the study. The body composition analysis was carried out by anthropometry measurements (skinfolds and circumferences) and BIA. The results indicate that the children who started ART or switched treatment had improved growth and FFM. Miller's (1993) study followed infants that were HIV infected and exposed in Boston, United States of America. The body composition analysis performed on the children was tricep skinfold and MUAC. It was found that the HIV infected child had significantly ($p=0.01$) less FFM than the control however both were within the normal range. Arpadi (2009) followed 64 HIV infected children and 147 uninfected children aged 6-16 yrs. Of the HIV infected children 94% were on treatment at the time of the study. DXA scans were done at baseline and yearly for two years thereafter to measure the body composition. The findings were that FM was similar between the groups but the overall distribution was different. When focusing on the HIV infected child they tended to have less leg fat and more

arm and trunk fat/. This discrepancy between the groups increased overtime and may predispose the HIV infected children to cardiovascular disease.

Although there have only been a few studies that examine and report on FFM in early childhood (34, 35), these studies have shown that FFM and clinical outcome are related (36). There is a link between a decline in quality of life, a decrease in FFM, muscle mass and immune system while there is an increased HIV viral load mortality and disease severity (6, 17).

The study of FFM in HIV infected infants and children can aid clinicians with the treatment of HIV and the improvement in their quality of life. FFM can model the tolerances and efficiency of antiretroviral therapy thus impacting on clinical outcome (36). As FFM has an effect on treatment out comes it would then justify that FFM be studied more closely as there is a high risk of loss of FFM during the disease process. It has been reported that antiretroviral treatment has increased FFM in patients and that it can help to established targeted nutritional support (36). There are two ways in which FFM can be measured firstly as a direct measure (DXA scan) and secondly as a product of muscle function (hand grip strength). Thus the ability to measure FM or FFM would enable a better interpretation and treatment of HIV disease.

1.1.4 Body composition models

There are a variety of techniques that can be used to study body composition such as Underwater weighing and Potassium counting but the majority of these are not practical in

a routine clinical setting as many require specialized equipment, trained staff, and are time consuming and expensive.

Broadly speaking all body composition techniques are based on the one of the four body composition models which divides the body into different compartments. Table 1.3 tabulates a summary of the different compartment models which are briefly described below.

Table 1.3: Compartment models for body composition

Two compartment model	Three compartment model	Four compartment model	Five level model
Fat Free Mass	Total body water	Body Cell Mass	Other
		Extracellular water	Blood
	Protein and minerals	Extracellular solids / (BMC)	Bone Skeletal muscle
Fat Mass	Fat Mass	Fat mass	Adipose tissue

The first, and still most commonly used, model developed was the two-compartment model which was developed in response to renewed interest in the links of disease risk and excess FM (20). In this model the body is divided into FM (weight of the entire removable lipids) and FFM (body weight-FM) (25, 37). In the two compartment model FM represents the storage of inactive energy in the form of adipose tissue (38) and FFM (also known as lean body mass) is defined as all the non-fat compartments of the body including cell mass, extracellular fluid, non-metabolizing proportion of the body which contains organic and inorganic compounds such as collagen, reticular, elastic fibers (39).

The two compartment model is based on the assumption that the composition (water and potassium) and the density of FFM are constant (4, 25). This however is not entirely true as there is inter-individual variation of composition of FFM and furthermore the chemical maturity of FFM is not achieved until a person reaches adulthood. In children a different constant is therefore used to make allowances for the differences in chemical components. In healthy, white, young adults the two-compartment model has however been found to be acceptable at estimating FM (40). There are however additional concerns that the underlying assumptions may not be valid for all sexes, races and disease states (40). Examples of techniques that use the two-component method are: Total body water (^{18}O isotope or tritium), Body density (Under water weighing (UWW), Total body potassium, Total body nitrogen, Urinary creatinine excretion, Bioelectrical impedance and Anthropometry.

The three-compartment model measures the density of fat, water and body solids to determine the body composition of healthy adults and older children (40). The model requires the use of both UWW and isotope dilution techniques which are more technically difficult than those used in the two-component model and are often impractical in the case of children (25). The combination of UWW and isotopic dilution results in the density, water and body solids being measured. One of the assumptions inherent in this model is the total protein and bone mass is constant. This model has not proved reliable for people with depleted body protein or bone mass. As a result a depletion of either of these would likely result in inaccurate calculations (40).

The four-compartment divides the body up into FM and FFM, with the FFM divided into extracellular water, body cell mass and extracellular solids/BMC. In order to measure the mass of the protein and bone mineral, neutron activation analysis (protein) and Dual energy x-ray (bone mineral) techniques are used (40, 41). Very few research centers worldwide have access to the neutron activation analysis technique while DXA is more readily available (40, 41).

The five-component model is regarded as the best model and is recommended if both the expertise and equipment needed to measure the different components of the five component model are available. This model is able to measure chemical and molecular structures which remain relatively constant in both the case of disease and health. As a result, calculating total body composition from the elemental level has fewer assumptions related to tissue density, hydration and structure making it more accurate and can even be used in the replacement of a wet chemistry assay, like cadaver studies (40).

1.1.5 Anthropometric regression equations

Regression equations which incorporate skinfold thicknesses and other anthropometric and demographic variables to estimate FM, FFM or BF% are the most feasible in-field technique and are recommended for day-to-day use (25). There are a number of reasons why anthropometric measurements are frequently used to assess the nutritional status of individuals and communities. Anthropometry is more objective than clinical assessments (42). Skinfold measurements provide an estimation of the amount of subcutaneous fat at precise points on the body and can therefore be used to track changes of FM deposits at these specific areas (2, 37, 43, 44). The most common anthropometric measurements taken

are weight and height, then mid upper arm circumference (as a screening tool for malnutrition) followed by a variety of skinfold thicknesses (25). These are relatively practical bed-side, non-invasive procedures which require only limited and easily portable equipment (3, 25, 45). The actual process of taking a skinfold measurement is relatively quick and easy once a person has been trained in the technique (44). Widely available reference data and tools make it relatively easy to identify departures from the norm with many studies having reported skinfold thickness and other anthropometric data on children of all ages from, infants to adolescents (44). Lastly, skinfolds are suitable for under-, normal- and moderately over-weight adults and children (2).

There are however some notable limitations to using skinfold thicknesses to estimate FM or BF%. The most important draw-back of skinfold methods is that subcutaneous fat measurements at specific points are used to estimate the BF% and/or FM of the whole body (44). The three major effects that limit the accuracy of the skinfold method are 1) measurement error, 2) physical properties of certain tissue and alteration in composition of the tissue 3) assumptions incorporated into regression equations when estimating body composition from anthropometric measurements (45).

The regression equations are based on assumptions. The first assumption is that the composition (water and potassium) and the density of FFM and FM remain constant (4, 25). The accepted values in adults are 72-73% for water and 2.66g/kg potassium for males and 2.55g/kg potassium for females. This however is not entirely true: as there exists an inter-

individual variation of FFM. When measuring body composition in children these constants are no longer valid as the chemical maturity of FFM is not achieved until a person reaches adulthood. Thus the underlying assumption could be affected by age, gender, race and disease state (40). Hence the uses of pediatric constants in regression equations designed for children (4, 25). A further assumption is that raw skinfold measurements can be used to predict BF% by using prediction equations. This allows for the inclusion of predictive error. Lastly the use of prediction equations uses theoretical assumptions that may add in additional errors (4).

The accuracy and repeatability of the measurements are largely dependent on the technical skill of the healthcare worker and their adherence to protocols (2, 25). In addition, the validity of skinfold thicknesses measurements in infants are not as good as in older children and adults.

Due to the possible inaccuracies of measurement, in a study setting, it is important to establish the reliability of anthropometric measurements - specifically skinfold thicknesses - by assessing the variability of measurements when measured, by the same researcher, on the same subject (45). Edwards (46) recommended that $\geq 66\%$ of the duplicate skinfold measurements should be within $\pm 5\%$ of each other in order for skinfold measurements to be regarded as reliable (43).

Regression equations developed for specific populations have been validated against other more sophisticated body composition techniques, most commonly a two compartment model technique. On the whole, population specific studies have shown that the use of skinfold thicknesses in estimating total body fat from regional subcutaneous fat correlates well with densitometry (44). The population specific regression equations focus on variables (participant's age, gender, population group and the skinfold measurements) that have been identified as factors that can affect the body composition component that the regression equation calculates. While there are a number of regression equations that can be chosen from depending on the variables identified (25), there are limited equations available, which calculate total BF% or FFM in young children aged less than 3 years (44). Unfortunately, there are very few regression equations for FM or BF% and none that estimate FFM, that have been developed from skinfolds, circumferences, weight or heights, for South African children. Cameron et al. (2004) developed a regression equation for South African children aged nine years. The equation was developed using DXA and skinfold thicknesses of children living in the Soweto area. However, to date, no regression equations have been developed for South African children infected or uninfected with HIV, aged 9-36 months.

The lack of appropriate reference data and a South African specific regression equations has resulted in the fairly wide-spread use in South Africa of equations that were developed in other populations (country, race, socio-economic status). Due to the limitations of existing regression equations it is not necessarily appropriate to use equations that were developed for a different population. This is because various population dependent factors such as race and socio-economic status may affect the assumptions and thus limit generalizability and validity of the regression equation in the new population (25). A further compounding factor

with the use of non-South African developed regression equations is that within the South African children population there is a large diversity in race. This may prevent the development of a country specific regression equation but rather the development of race dependent regression equations that are developed for South African children. This concept is supported by the International Society for Clinical Densitometry (ISCD) official position that race as a key factor when reporting or comparing body composition results (47). Other considerations that can affect the body composition of an infant or child are and would also need to be taken into consideration when developing the regression equations are:

- duration of breastfeeding and appropriateness of complementary foods as this may influence the amount of FFM and risk of obesity later on in life (35).
- Maternal factors like maternal age, pregnancy BMI, height, education, social class, smoking during pregnancy may affect FFM at 12 months of age and the weight gain in the first year of life (34).
- Infant birth weight (34)
- Rapid birth weight in the first 6 months of life (34) as this may be associated with overweight and obesity later on in life and increased trunk fat.

One of two actions is needed when there is an absence of appropriate regression equations for the population group under study. Firstly, regression equations (from similar populations) should be validated in the population of interest prior to their use. Secondly, if the regression equation from other populations could not be validated, a new validated regression equation specific to the study population should be developed.

In practice, however, when published regression equations developed for other populations are applied to a new population, a validation study is not always conducted. In the literature there is a blanket recommendation to use the regression equations proposed by Slaughter et al. (1988) or Dezenberg (1999) when measuring BF% in black African children (48). As an illustration of this, Monyeki (49) conducted a study of 855 urban South African children aged 7-14 years. In this study BF% and FM were calculated using Slaughter et al. (1988) regression equations for both males and females. The rationale for use of these equations was that they had been internationally accepted for use in different ethnic groups (50). However, when Cameron (48) tested the Slaughter et al. (1988) and the Dezenberg (1999) regression equations it was found that both equations significantly underestimated ($p < 0.001$) the amount of FM when compared to DXA in black South African children. As a result Cameron does not recommend the use of these equations in FM predictions for black South African children aged 9 years (48). This result highlights the importance and necessity of completing validity tests on any regression equation that has been developed for a different population when being used in a new population.

While there are no published regression equations that use sub-scapular and/or triceps skinfold for HIV-infected or HIV-uninfected South African children between 9 and 36 months of age, there are equations that have been developed in other populations that have one or two similarities to this study's population with regard to age, socioeconomic status, disease state or race (44, 48, 51, 52). In order to validate these regression equations they need to be compared to a gold standard or an acceptable criterion method for measuring FM, FFM and/or BF%.

1.1.6 Gold standard and criterion methods

A “gold standard” is the measurement technique that has been established as valid, credible and reliable and that has been accepted as the best technique for that specific age group. The gold standard for measuring body composition has been established as direct chemical analysis of the whole human cadaver (4, 25). Chemical analysis of fetus and infant cadavers was started in 1923 by Moulton (40). Data from this direct analysis method have been used as the reference standard for the development and validation of other body composition assessment methods. There is, however, no complete set of chemical cadaver data available on body composition from infancy to adulthood. Between infancy and adulthood, only a single analysis exists of a 4.5 year old child who died of tuberculosis meningitis. In 1963, a body composition study was conducted on infants of diabetic mothers. A small number of studies were conducted on adults from 1950 - 1980 (40). Thus far, the analyses from cadaver studies have shown that while the weights of specific organs are similar between individuals, the chemical makeup of the organ tissue is not constant from birth (40).

As no in-vivo gold standard is available, a number of criterion methods have been developed including UWW, Total body water, DXA, Whole body counting and Neutron Activation Analysis (NAA). These have been accepted sufficiently accurate criterion methods in adults and are used to validate new techniques of measuring body composition (4).

Despite the variety of techniques available, no single technique is suitable in all circumstances (age, gender, ethnicity, sexual maturation, disease state, physical activity or in field application) due to the specific limitations of each technique (25, 37). Thus a thorough understanding of the limitations of each technique is required before an informed decision can be made as to which technique is appropriated for use as a criterion method in the population of interest. All methods used to measure body composition, apart from cadaverine methods, are indirect. For example UWW uses the direct measurement of the volume of water displaced by the participant to calculate the BF%. Once the volume of water has been measured, the density of the body (D_b) is then calculated with an equation that incorporates the volume of displaced water, the participant's underwater weight and their scale weight. Within this method there is a limitation in obtaining an accurate body volume as the participant's residual lung volume needs to be subtracted from the displaced volume of water measured. The equation ($1/D_b = f_{fat} / D_{fat} + f_{FFM} / D_{FFM}$) is used to calculate the participants FM and FFM, where D_{fat} is the density of fat and D_{FFM} is the density of FFM and F_{fat} is the fat mass. This equation uses the assumption that D_{fat} and D_{FFM} are relatively constant. Further assumptions in the calculation of BF% in this method include assumptions related to body hydration, protein and mineral content (40).

A further complication in infants and children is that both the constants and coefficients of many variables are different to that of adults. Once a person has reached adulthood, it is assumed that their FFM composition is constant (41). The accepted FFM constants in adults are assumed to be 73% water; potassium content is 2.66g/kg in men and 2.55g/kg in women (25). However during infancy there is a period of rapid growth where dramatic changes occur

in the composition of the body with each of the components of the body (mineral, protein, water and lipid) following a different linear growth rate (40). Children have a lower density mineralization and potassium content and higher water content when compared to adults (25). By using the pediatric constants instead of the adult constants some systematic bias is removed from the pediatric composition models however residual age related bias is likely. In addition, there are limited body composition reference data for healthy infants and toddlers (one - four years). Two main reasons cited for this are: the difficulty of determining measurements due to difficulty in conducting some of the methods in children as these often require children to follow complex instructions and the limited accuracy of the instruments used to measure small body sizes (40).

Reilly (25) developed criteria to be used to assess whether a body composition technique would be a suitable criterion method with in a study population. The technique chosen as the criterion method should be one where the measurement errors are acceptable and quantifiable and thus able to be used to validate other methods (53). The following criteria, as laid out in table 1.4 can be used to determine whether a method would be suitable for a pediatric population study.

Table 1.4: Criteria for choosing a body composition method in children (25)

Practical issues	<ul style="list-style-type: none"> • Is it acceptable in children? • Is it affordable? • What is the training requirement for staff? • Is the method safe?
Validity	<ul style="list-style-type: none"> • Is the method valid?

	<ul style="list-style-type: none"> • What are the assumptions made by the method? • Are the assumptions true for children? • Has a validation study on this method in children been published? • Was the validation study done correctly? • What was the error in the method and does it meet acceptability criteria? • Was the method valid for groups or individuals? • Does the method have cross validity?
Precision	<ul style="list-style-type: none"> • How precise is the method? • Does it detect changes in body composition? • Is it adequate for groups or individuals?

1.1.7 The use of DXA as a criterion method in children

DXA scans were developed by Mazess in 1981 to measure bone mineral content, FM and FFM (54). This technique is classified as a three compartmental model (40). The initial DXA method had two limitations: it used radioactive material and had an error in the calculation of bone mineral content. This error was due to the assumption that the composition of the non-bone tissue that overlapped with the bone was constant while it does vary. The DXA method has since been corrected and no longer uses radioactive material. Instead, it makes use of an x-ray tube behind a filter. Consequently, it allows the measurement of the proportions of BMC, FM, FFM and BF% of a body (54).

The annual regulatory limit of radiation received by a child who is younger than 18 years old in a clinical study is limited to 0.1mSv per year. To date there has been no reports on the associated risks due to the exposure to radiation during a DXA scan (55). In fact the amount of radiation that is absorbed during an infant whole body scan using a Hologic DXA machine

is approximately 0.012mSv. This is significantly less than a transatlantic flight of 0.08mSV and even less than daily exposure to natural background radiation of approximately 3 mSv per year. This means that one DXA whole body scan is comparable to less than 1 day of natural background radiation (56).

Although DXA scans are not generally regarded as a gold standard in the literature, it is widely accepted as a suitable criterion method in children (53, 54). The reason for DXA not being regarded as a gold standard is that in populations that have altered hydration status (age, maturation and disease state), DXA may not measure their FM and FFM accurately (57). This is because DXA incorporates an assumption that the hydration of FFM is uniform and fixed at 0,73mL/g and hydration has been found not to be constant in the elderly, very young or sick. Abnormal hydration can affect the child's R_L , this can result in an error in the calculation of the lean tissue credited to each pixel. As fat and lean tissue are calculated as the sum of the total weight, the amount of fat will also be incorrectly reported. A second reason that limits DXA's use as a gold standard in the case of children, is that it has been found to be less accurate as total body weight decreases (53). A third assumption when DXA is used to calculate body composition is that the soft tissue layer over bone has the same fat to lean ratio as other non-bone pixels in the same scan (40). Despite DXA being commonly used in the cross validation of other body composition techniques and prediction equations, it still requires further research and standardization before it can be used as a gold standard (37, 40).

The evidence that DXA can be used as a criterion method in children is outlined below using Riley's criteria in Table 1.4.1.

Table 1.4.1 Table of evidence to use DXA as a criterion method in children

Practical	It is generally safe in children and is often used in research (58).
	DXA is generally considered more acceptable and safer than Magnetic Resonance Imaging or Computed Axial Tomography scans as it is less expensive and uses less radiation (41).
	While it is safe and quick it does require that participants sleep or not move during the scan (37). This can be a practical disadvantage as it can be time consuming and difficult to get children to fall asleep.
	DXA scans are able to divide the body up into regions or segments, which allows one to monitor changes in the distribution of BF% between different regions (37).
Validity	Against multi-component models, DXA has good agreement (37).
	It has also been found to be highly reliable and to have good agreement with hydro densitometry (37).
	The accuracy of measuring BF% with DXA scans is reported to be 1.2% or within 3% (37, 39).
Precision	There is an acknowledgement that DXA is considered as an accurate method, specifically in children older than 4 years old (4) with some even recommending it as a potential gold standard method (37).
	DXA scans are recommended to monitor the changes in fat redistribution (lipodystrophy), a side effect of some antiretroviral agents, as DXA can track changes in the FM in arms, legs and trunk. However scans cannot detect wasting of the face (59).

When DXA is used as a criterion method it needs to be acknowledged that DXA has been reported to overestimate FM in early infancy (60). The software and the machine model have to be included in the methods as body composition results of individuals can vary if equipment from different manufacturers has been used (2, 40).

It is important to report whether the head was included in the analysis presented or not, so that the results can be compared to other studies that have either included or excluded the

head. The International Society of Clinical Densitometry recommends that the head should be excluded from the total body analysis when reporting on children (61). The American National Health and Nutrition Survey (NHANES) have used DXA sub-total body measures (excluding the head) in their data base of 8-20 year old children (61). The reason cited for this that the preferred skeletal sites for DXA scans are the lumbar spine and the total body (excluding the head). The head is excluded as it makes up a large proportion of the total bone mass but changes little with growth, activity and disease. If the head is included in the analysis it may hide losses of bone at other skeletal sites. (55, 61) (54). The focus of this study is not on bone density however the head will not be included as the results will be published.

An important consideration when working with children that are infected with HIV is that changes in hydration can affect the accuracy of the scan. DXA scans are however less affected by changes in TBW content than hydro densitometry and hydrometry methods (39). However, DXA scans have been used in adults with HIV and the technique has successfully measured the changes of FFM in the patients that had wasting (39).

After considering the practical, validity and precision criteria it was decided that the DXA absorptiometry method would be the most appropriate criterion method to compare regression equations from skinfold thicknesses in South African children that are infected with HIV. A further advantage of DXA is that it would also be able to describe the amount and layout of FM, FFM and BF% detected in our study population.

1.1.8 Identification and selection of published regression equations to be assessed

The table below lists the equations selected for the study to be tested for agreement with DXA. The reasons why the four regression equations were chosen to be included in this study are as follows. Firstly, general guidelines recommend the use of Slaughter (1988) (62) and Dezenberg (1999) (51) regression equations in children of African descent (48). As the Slaughter (1988) (62) equation was developed for prepubertal children of African descent and Dezenberg's (1999) (51) regression equation was specifically designed for children with a mixed ancestry. Skaikh and Mahalanabis's (2004) (52) equation was included because of the children's young age and that they were also from a developing country. The Goran (1996) (63) equation was chosen because of the younger age of the children. Secondly at the time of writing the parent study protocol due to the general guidelines [to use Slaughter (1988) and Dezenberg (1999) regression equations] the only skinfolds that were of interest at the time to study body composition was tricep and subscapular. Thus all four of the equations have a combination of factors including- subscapular and tricep skinfolds, MUAC, weight, age and sex.

Table1.5: Table of regression equations used for comparison to DXA scans

Equation name	Measured	Country	Equation	Population Tested	Sample size	Ethnicities in study	Derived from	Age group of participants
Slaughter (1988) (62)	BF%	USA	$BF\% = 1.21 \times (T + S) - 0.008 \times (T + S)^2 - 3.2$	For black males (Tanner 1)	310 66 prepubescent 59 pubescent 117 post	Caucasian Black (number of participants for each	Body density from underwater weighing, BMC from photon	8-25 year olds but can be used in younger
			$BF\% = 1.33 \times (T + S) -$	For all females				

			$0.013 \times (T + S)^2 - 2.5$		pubescent 68 adults	race not given)	absorption & total body water derived from deuterium oxide	children
Shaikh & Mahalanabis (2004) (52)	BF%	India	Boys; %fat= $5.304 + 0.269 \times T + 0.50 \times S + 0.685 \times M - 0.063 \times A$ Girls; % fat= $7.017 - 0.053 \times T + 0.201 \times S + 0.765 \times M + 0.052 \times A$	Indian Children from urban low socioeconomic status	184 children	184 Indian children	BIA and skinfold measurements total body water derived from deuterium oxide	Boys: 26.8 months with a SD of 14.8 months Girls: 30.2 months with a SD of 15.82
Dezenberg (1999) (51)	FM in g	USA	FM(kg)= $0.342 \times W + 0.256 \times T + 0.837 \times \text{sex}$ (1=boy, 2= girl) - 7.388		177	104 Caucasian 69 African American 4 Native American	DXA	4-10 year olds
Goran et al. (1996) (63)	FM in g		FM= $0.23 \times S + 0.18 \times W +$	Male and female	50 prepubescent	46 Caucasian children	DXA	4-10 years

			$0.13 \times T - 3.0\text{kg}$			4 Native Ameri can		
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Key: T= triceps skinfold thickness (mm), S= Subscapular skinfold thickness (mm), M= mid arm circumference (cm), A= Age (months), W= Weight (Kg)

However, both the Slaughter (1988) and Dezenberg (1999) equations have limitations. These limitations need to be explored in order to understand why they might or might not predict BF% in our population group accurately. Firstly, neither equations correctly used Tanner staging to identify children who were prepubertal (48). Both Slaughter (1988) and Dezenberg (1999) incorrectly categorized their prepubertal groups of children. According to their methods the children were classified as prepubertal however some of the children were classified as Tanner 2, which in fact indicates that they had already started puberty. This indicated a fault in the classification. Furthermore in the Dezenberg (51) study, it states that the Tanner classification was not assigned to all their participants but rather the assumption was made all the children were prepubertal, thus the person who assigned the children to the prepubertal group may have entered error to the group (48). The Slaughter study did not indicate how many black participants were in the study. It only specified that the study included 50 male and 16 female children of mixed ethnicity. The Dezenberg (51) African American sample size was made up of 31 boys and 38 girls (48).

Goran (63) did not make use of the Bland-Altman method to assess if the skinfold thicknesses and DXA had similar results. In his study Pearson correlation coefficients were used (63). This may have affected the reliability of the agreement as the incorrect statistical

method was used possibly resulting in an overestimation in the agreement between the two methods.

1.1.9 The description of body composition in the HIV-infected child.

South Africa has a high burden of HIV disease. The number of HIV-infected children under the age of 15 years in 2008, was estimated to be 220 000 (high low estimates of 130 000-300 000) by the United Nations Joint Program on HIV & AIDS (UNAIDS) and 340 000 (high low estimates 230 000 to 450 000) by the Human Science Research Council (HSRC) (1). The estimated number of children that needed highly active antiretroviral therapy (HAART) was pegged at 91 000 (high low estimate of 52 000 to 130 000) (1). In order to effectively treat and manage children with HIV, a good understanding of how the disease effects children's growth and body composition is needed.

Body composition reference ranges of healthy children are however required in order to understand the relationship between HIV and body composition in children. Unfortunately, there is a lack of reference data for FM, FFM and BF% for healthy children from 9 months to three years old. The lack of FM, FFM and BF% data in children aged nine months to three years could be the result of the following: the lack of an available and practical method for measuring body composition in this age group, the difficulty of taking measurements in this age group as younger infants sleep more easily and older children can lie still for DXA, and the high cost of equipment needed for the criterion methods of measuring body composition in children. Furthermore, differences in body composition between races and rapid growth

at this age results in issues of generalizability and therefore generation of age and race specific reference data (40).

1.1.10 Published body composition FM, FFM and BF% values for children

Published literature was searched for studies that explored body composition in children that had reported DXA results. A standard literature search using PubMed including Medical Subject Heading terms) and Google scholar were used. A combination of the following words were used: body composition methods, fat mass, fat free mass, body fat percentage, HIV, skinfold regression equations, subscapular, triceps, DXA, pediatric, toddler, preschool and infant. Only two publications were revealed. The first publication included data partially stratified by race, sex and age-group: White/ European-American (n=145), Black/ African American (n=78) and Hispanic/ Mexican American (n=74) children and aged in groups: 3-5 years, 5-9 years, 10-14 years and 15-18 years. The results reported were BMC (g), FFM (g), FM (g) and BF% (40). The limitations of these data are that age groups did not actually include children <3 years of age and that the number of children per age group of each race group was not indicated precluding comparisons of means and standard deviations. Furthermore, the Ellis study did not disclose whether the head measurement was included in the DXA scan results or not. In addition the specific software used was not recorded. As a consequence of the limitations of the results, data from the Ellis study could not be used to compare with data from this study.

The second set of published DXA results was published by Shypailo et al. (53). The focus of this study was to compare the differences reported in DXA results of children aged 1-15 years when the same machine was used but with different software versions (Hologic version 12.1 and 11.2). The focus of this study was not to describe the body composition of the children but rather to compare and describe the difference in results when different versions of software were used to analyse DXA data. The description given of the methods and children used in this analysis indicated that the DXA data had been collected from a variety of previous studies and that health status and race had not been available. The study reports body composition data on 6 male and 11 female children aged 1-3 years.

Table 1.6: DXA results as reported by Shypailo

Children 1-3 years	N	Total Body Fat Mean and SD	BF% Mean and SD
Male	6	3.26 ± 0.5	24.9 ± 3.4
Female	11	4.36 ± 0.8	34.4 ± 7.5

As the aim of Shypailo's study was to compare the DXA results between two different versions of software, there was no explanation given as to why there was substantially higher total body fat and BF% in girls. While absence of data on race and health status does somewhat limit the interpretation of this data this is the best available data on DXA scan results in this age group.

1.2 Overarching goal of this study

There is evidence that the inclusion of body composition studies may benefit the management and understanding of HIV disease progression. Detailed information is needed on the amount and patterning of FFM, FM and BF%. However, the criterion methods for measuring body composition are either too expensive, technical or not sufficiently practical in children for them to be implemented in routine clinic settings. There are no published regression equations for estimating BF% or FM for young children which have been developed for the South African paediatric population. Regression equations which were developed in other populations are currently used to estimate BF% or FM in South African children infected and uninfected with HIV despite these not been validated in these populations. Validation of these equations is clearly necessary. In order to validate a body composition measurement such as regression equations, the validation must compare the method with a well-known criterion method that consists of at least a two body composition component method (25). In order to be used the method should have suitable agreement with the criterion results. If two methods have high levels of correlation it does not necessarily mean that the new method can be used as a reference method as correlation does not indicate if they are in agreement (25). Agreement between the two methods can be assessed by using Bland-Altman methods (25).

In order to validate the existing regression equations data from a criterion method, such as DXA, is required. The overarching goal of this study is to describe the body composition of the HIV infected study population and validate the existing regression equations in a

population of HIV-infected South African black children 9-36 months of age and secondly to describe the children's body compositions.

1.3 Objectives

1.3.1 To describe the demographics of the study population.

1.3.2 To describe the FM, FFM and BF% in young, black, HIV-infected SA children when they start ART

1.3.3 To assess the agreement between BF% and/or FM calculated from four regression equations using combinations of subscapular, tricep, weight, age, sex and MUAC and the BF% and/or FM calculated from DXA.

2 METHODS

2.1 Study design

This is a retrospective, observational, cross-sectional study which includes descriptive and comparative analysis of baseline data collected as part of a study “Body compositional changes in a cohort of HIV-infected children receiving antiretroviral therapy in a population where background malnutrition is prevalent (R14/49 Egbers)” which is from here forward referred to as the parent study.

The author of this thesis was involved in the parent study with regards to: identification of possible participants, informed consent and enrollment, anthropometric assessment and monitoring of DXA scans, participant retention and booking of visits, team meetings and writing of body composition related protocols and monthly reports on the study’s progress and statistics.

The inclusion of a HIV-uninfected but exposed control group of the same age, race, social economic background would have resulted in a better understand of this study’s findings. However this study is a secondary analysis of the parent study. The parent study did not have a control group and thus it was not possible to have one for this study

2.2 Setting and participants of parent study

2.2.1 Geographic details

The parent study took place at Harriet Shezi children's clinic (HSCC) which is a public sector, paediatric HIV clinic based in the tertiary level academic Chris Hani Baragwaneth hospital in Soweto. The clinic is situated in an urban setting but serves both urban and semi-urban communities due to its large referral catchment area. The parent study ran from October 2007 until November 2008.

2.2.2 Sample size

The available sample size for the current study was determined by the parent study. DXA scan data were required for children to be included in this analysis. Of the 46 children enrolled in the parent study, 37 children had DXA scans performed prior to commencement of HAART and data from these children were included in this analysis. The inclusion criterion for this study was that the participant needed to have been included in the parent study's baseline data. Below is a description of the selection criteria for the parent study.

2.2.3 Inclusion and exclusion criteria

The inclusion criteria for the parent study were children:

- between 9-36 months of age at enrolment;
- meeting criteria for starting antiretroviral therapy as per the South African guidelines (64);

- antiretroviral treatment naïve. Children who received a single dose of Nevirapine (NVP) given at birth for prevention of mother to child transmission were defined as treatment naïve; and
- confirmation of HIV positive status of the child

Children were excluded from the study if:

- their guardian or parent did not want the child to participate in the study
- they were not eligible to start HAART; or
- they had any pre-existing conditions that would have affected their body water status.

For example children with oedema or hydrocephalus were excluded from the study.

In addition to the above, children were excluded from this analysis if they did not have a baseline DXA scan.

Written informed consent for the parent study was obtained from parents or legal guardians. Once the participant had completed the informed consent process, they were given a return date. This was the date on which the child was expected to commence HAART. According to the study protocol, their DXA scan, anthropometric measurements and bloods were to be recorded and documented on the same day. In order to allow for rescheduling of the DXA scan in the event that the child did not sleep the DXA scans could be taken within a 10 day window period either side of the ART start date.

The South African Guidelines for the initiation of HAART in children was followed by the health care team (64). The children were seen by the health care team and received counseling on taking the HAART medication and nutrition. The parent study “Body composition changes in a cohort of HIV-infected children receiving antiretroviral therapy in a population where background malnutrition is prevalent (R14/49 Egbers)” was given approval from the Human Research Ethics Committee (HREC): Medical committee dated M060423 (Appendix 2). This study was given ethical clearance by the same committee-clearance certificate M090807.

2.3 Variables and data sources

2.3.1 Descriptive variables

The following variables were included in this analysis: gender, race, age (months), HIV WHO stage (3&4), TB treatment, PMTCT exposure, immunization coverage, the child’s developmental milestones (appropriate or delayed), CD4%, CD4 count, HIV viral load, hemoglobin, ALT, cholesterol and triglycerides.

2.3.2 Anthropometric equipment and measurements

The anthropometric variables that were collected in the parent study were weight (kg), height (cm), head circumference (cm), mid-upper-arm-circumference (cm), tricep skinfold (mm) and subscapular skinfold (mm).

2.3.2.1 Anthropometric equipment

The following equipment was used when taking the anthropometric measurements:

- Weight (kg) was measured using a Scale 2000 infant scale or SECA (2 in 1) scale.
- Recumbent length (cm) of children < 2 years was taken using Seca infant mat.
- Height (cm) was measured with a Holtain stadiometer.
- Skinfold measurements (mm) were taken using a Harpenden skinfold caliper.
- The MUAC (cm) was measured with a non-stretch measuring tape.

2.3.2.2 Anthropometric methods

According to the parent study protocol the anthropometric measurements were taken in a private room. This room was heated in winter to improve the comfort of the participants. All measurements were taken twice unless they varied substantially different, (more than 1 cm for height or length and 0.5cm for skinfold thicknesses). Triceps and subscapular skinfold thicknesses and MUAC were measured using the standard landmarks and techniques. All measurements were taken on the left hand side of the body. For the skinfold measurements in this study to be considered reliable, 66% of the duplicate measurements would need to be within 5% of each other (43).

The majority (>85%) of skinfold measurements were taken by the study dietician. However, five out of the 37 measurements were taken by a stand in dietician, trained by the study dietician. The calipers used to measure the skinfolds were returned to zero daily and calibrated on a yearly basis. The scale was checked on a weekly basis by the use of

certified weights which were weighed to see that the scale measured the same weight as the known weights. The stadiometer was also calibrated weekly to see that it correctly measured a set length of 60cm. If any error was detected the instruments were sent for repairs.

Procedure for weighing infants 9 – 12 months infants (65)

- The scale was zeroed before the weight was taken.
- The infants were weighed naked. The mothers were asked to undress the children.
- The weights were only read once the infant was calm. Thereafter the weight was record in the source document.

Procedure for weighing children older than 1 year (65)

- Children were weighed while wearing the minimum of clothing.
- The children were positioned in a standing position on the scale with feet apart and looking straight ahead.
- If a child was not able to stand unaided, the 2 in1 function of the scale was used to weigh the child whilst being held by the mother.

Procedure used for measuring recumbent length in infants/children unable to stand (65)

- Recumbent length was taken from the crown to the heel of a child.
- The mat was smoothed, to ensure it was flat before each measurement was taken.

- Outer clothing including shoes, socks, hats and hair ribbons or clips was removed. The infant was placed on the measuring mat, facing upwards, with their head towards the fixed end and body parallel to the long axis. The shoulder-blades rested against the surface of the mat. The examiner then applied gentle traction to bring the crown of the infant's head into contact with the fixed headboard. The mother was then asked to hold the child's head in this position.
- The examiner held the child's feet. Toes were pointed upwards, the child's knees were held straight and the moveable footboard was positioned against the heels.
- The reading was taken to the nearest half a centimeter and recorded in the source document.

Procedure used for measuring heights of children able to stand (65)

- All outer clothing was removed; shoes, socks and hair accessories.
- The children were asked to position their head in the Frankfurt plane position. The head did not need to be in contact with the vertical surface.
- The children's feet were then placed together with their knees straight and their heels, buttocks and shoulder blades firmly placed on the stadiometer.
- The child's arms were allowed to hang loosely at their sides.
- The children were asked to take a deep breath and stand up straight as the moveable headboard was lowered until it just touched the crown of the head.
- The measurement was taken and recorded in the source document.

Procedure used for measuring head circumference (65)

- The infant's/child's head was positioned so that the line of vision was perpendicular to the body and in the Frankfurt plane.
- The tape was placed, level, just above the supra-orbital ridges around the most prominent part of the frontal bulge, and over the part of the occiput that gave the greatest circumference.
- Measurement was taken and recorded in the source document.

Procedure used for measuring MUAC (65)

The children were asked to stand or held by their mother/caregiver in a relaxed position with their arms hanging by their sides.

- First the acromiale was marked. This was identified by:
 - Palpating along the top of the scapula to the corner of the acromion.
 - Once a dent (which indicates where the bones join) was found it was marked which indicated the most lateral aspect.
- Secondly the radiale was marked and identified by:
 - The dimple of the right elbow was palpated in order to identify the space between the humerus and the radius.
 - The most lateral part of the radial head was marked
- The mid-acromiale-radiale level was marked mid-point between the two marks.
- The circumference measurement was taken at the mid-acromial-radiale level

- Constant tension was applied to the tape by ensuring that there was an indentation of the skin, while making sure that the tape still held its place at the designated landmark.
- The measurement was read and captured in the source document.

Procedure used for measuring skinfold thicknesses (65)

The following general considerations were taken in order to accurately measure the skinfold thicknesses:

- The child was asked to stand (or sit on their mothers lap, if very young), with their left arm hanging freely by their left side.
- The skinfold caliper was returned to zero before each measurement was taken.
- A make-up pencil was used to mark all skinfold landmarks.
- The skinfold was picked up and read at the marked line.
- The examiner used her left thumb and index finger to grasp and raise the skinfold and the underlying subcutaneous adipose tissue.
- Care was taken to ensure that no underlying muscle was picked up and mistakenly included in the skinfold measurement.
- The face of the caliper was placed 1 cm away from the edge of the investigators fingers but at mid-fingernail length.
- The caliper was held at an angle of 90° to the surface of the skin at all times.
- The raised skin was held with the left hand while the measurements were taken.

- The measurement was recorded after 2 seconds, when the full pressure of the caliper was applied.
- Procedure used for measuring the tricep skinfold thickness. The triceps skinfold site was identified as the posterior part of the triceps, in the mid-line, at the level of marked mid-acromiale-radiale landmark.
- The identified skinfold site was marked, measured and recorded.

Procedure used for measuring the subscapular skinfold thickness (65)

- The subscapular skinfold site was identified as 2cm along the line running laterally and obliquely downward from the subscapular landmark at an angle of 45°. The line of the skinfold followed the natural fold lines of the body (66).
- In order to locate the subscapular skinfold site the inferior angle of the scapula was palpated. The site was marked with a make-up pencil.
- The skinfold site was located 2cm laterally down from the subscapular at a 45° angle. The measurement was taken and recorded.

2.3.3 DXA equipment, measurement procedures and variables

2.3.3.1 DXA equipment

A DXA (Hologic, USA, Discovery W model S/N 71201, version 12,5:7) whole body scan was used to scan all children. The scans were operated fan-beam mode with a switch-pulse dual energy of 100kVp/140kVp. All the scans were analyzed by the computer software (Auto whole body version 12,5:7) provided by the manufacturer. The machine

employs automatic, continuous calibration using Hologic's patented Automatic Internal Reference System. In addition daily scans of known skeleton samples were performed for calibration.

2.3.3.2 Method used to administer DXA scan (67)

DXA scans were performed at the Chris Hani Baragwaneth, Birth to Twenty Unit, by an experienced DXA technician. When the infants were scanned, the first attempt used the "feed and sleep" method. Children who did not fall asleep were given a mild sedative as per the approved protocol. Children under the age of 24 months were given oral midazolam 0.2mg/kg per dose up to a maximum of 15mg. Children older than 24 months were given oral trimeprazine 2mg/kg as a single dose. Dose of trimeprazine was escalated to a maximum dose of 4mg/kg trimeprazine, only if necessary.

All clothing that contained metal was removed and children were scanned while wearing a minimum of clothing. Children were scanned in the standard supine position with their arms slightly bent at their sides and their legs bent with their feet almost touching. If needed; a soft pantyhose band was used to keep the feet in position and a small blanket was used to help position the head in the upright position. The scan time was approximately 6 minutes. Only one DXA measurement was taken unless the child woke up or moved during the scan in which case it was repeated. The DXA machine was calibrated daily.

Body composition, as measured by DXA scans, was expressed in FM (g), FFM (g), BF% and bone mineral content (g) with arms, legs, trunk and head regions of the body subdivided into these compartments. The DXA scan variables that were directly reported by the DXA scan output were BF%, subtotal FM (g), right and left arm fat (g), right and left leg fat (g), trunk fat (g), right and left arm lean (g), right and left leg lean (g), trunk lean (g) and BMC. The total mass as calculated by the DXA scan equaled the sum of FM, FFM and BMC. The bone mineral content of the participants will not be discussed in this study as the study is only focusing on FM, FFM and BF%.

In addition the following variables were derived from the DXA data:

- Arm fat (g) = right arm + left arm
- Leg fat (g) = right leg + left leg
- Extremity fat (g) = arm fat + leg fat
- % FM in arms = arm fat (g) / subtotal fat mass (g) X 100
- % FM in legs = leg fat (g) / subtotal fat mass (g) X 100
- % FM in trunk = trunk fat (g) / subtotal fat mass (g) X 100
- Extremity fat % = (arm fat (g) + leg fat (g)) / subtotal fat mass (g) X 100
- Trunk to extremity FM ratio = trunk fat (g) / extremity fat (g)
- % FM of arms = arm fat (g) / arm mass (g) X 100
- % FM of legs = leg fat (g) / leg mass (g) X 100
- % FM of trunk = trunk fat (g) / trunk mass (g) X 100
- Subtotal fat mass adjusted for height (g/cm): subtotal fat mass(g)/height(cm)

However, since some publications for example Shypailo (53) do not indicate if they included the head in the DXA results, the total BF% including head measurements was also used to compare results with the regression equations.

2.4 Data management

The descriptive and anthropometric data was obtained from records in the patient's file. The DXA data was collected from the DXA scan printouts. All data was entered by means of a single entry into Microsoft Access 2000 study specific data base. The information was then exported to STATA version 9.2 ®. The data was assessed for reliability using internal validity checks and plausible ranges and graphed to identify outliers.

In order to calculate the FM and BF% for the regression equations, the anthropometric data was inserted to the 4 different equations (Slaughter 1984, Denzenberg 1999, Shaikh & Mahalanabis 2004 and Goran 1996) as shown in Table 1.6 in the literature review. There were separate equations for boy and girls for Slaughter (1984), Dezenberg (1999) and Shaikh & Mahalanabis (2004), which were calculated according to sex to estimate the FM or BF%. However the estimated BF% or FM was combined for each regression equation for the scatter and Bland-Altman plots. The anthropometric data was also analysed by the WHO Anthropometric calculator v 2.0.2. The results were used to categorise the participants into the WHO Z score categories of WAZ, HAZ, and WHZ.

2.5 Bias

There was a degree of selection bias during the identification and screening of possible participants for the parent study as there was another study being run concurrently at Harriet Shezi Clinic where HIV-infected children, of the same age but without TB, were being enrolled. Since the presence of TB was not one of the exclusion criteria for the parent study, children without TB were preferentially enrolled in the latter study, whilst children on TB treatment were enrolled in the parent study. This resulted in only 3 children that were not on treatment for the TB included in the parent study. Not all of the children had bacteriologically confirmed TB.

Another form of selection bias which was encountered in this study was the requirement that the children had to have a parent or an adopted caregiver give their informed consent for the study. Due to the nature of HIV/AIDS many of the clinic's children are orphans. There is likely to be an association between orphan status and nutritional status resulting in the orphaned children having a different nutritional status to those who had parents. While it is possible to obtain individual waivers for this requirement from HREC, this process was not implemented for this study.

No pre-screening records were kept from the parent study. Therefore it was not possible to verify whether the children who were screened for the study and not enrolled or refused to participate were in any way different from the children who were enrolled.

2.6 Statistical methods

Data was imported into Stata v11.0 (Statcorp). Missing data was identified. Data from children with key missing data were compared to children with complete data using demographic, clinical characteristics and outcomes of interest to assess whether there was evidence of whether data were missing not at random. Outliers and implausible values of quantitative variables were identified and checked against source documents. Anthropometric values for each visit were calculated for each child as the mean of the two measurements most in agreement. After the data was cleaned, reliability of anthropometric measurements were assessed using Lin's concordance correlation coefficient.

The distribution of quantitative variables was assessed using quartile plots and tests for skewness and kurtosis. As a result of the small numbers of subjects and the finding that a fair proportion of variables were not normally distributed, medians and IQR were used to further describe the data. Wilcoxon rank sum tests were used for comparisons of unmatched data. Bland-Altman plots had 95% limits of agreement. Z-scores for anthropometric variables were calculated in Stata v11.0 (Statcorp) using the WHO 2006 growth standards and the WHO 2007 reference ranges in addition to ado files downloaded from the WHO website.

2.4.1 Rationale for use of Bland-Altman method

The Bland-Altman method was used as the statistical method to test agreement between two methods of measuring FM or BF%. Bland-Altman is appropriate for situations where there

is no available gold standard as it allows for error in measurement to be equally apportioned to both methods. Firstly, the results between the two methods were plotted against each other to determine the degree of agreement. This only revealed the strength of relation of the measurements and not the agreement. The Bland-Altman method plots the difference of the methods against the mean of the two methods in order to assess the agreement of the two methods (68). Bland-Altman methods can also determine if there is an association between measurement error and the mean value of the two methods. If there is no obvious relation between the difference and the mean, the bias is calculated by means of an estimation of the mean difference and the standard deviation of the differences.

When using the Bland-Altman method the limits of agreement are used to describe the distribution of the difference of the two measurements by the mean of the measurements. The limits of agreement indicate the standard deviation when the distribution is normal. If the intervals of the limits of agreement are wide it indicates that there may be a small sample size or that there is a large variation in the differences of the measurements, which indicates poor agreement.

2.4.2 Comparison between the sexes

Results were compared by sex despite the small sample size. The rational for the comparison of the sexes is firstly that Slaughter (1984), Dezenberg (1999) and Shaikh & Mahalanabis (2004), all have sex-specific regression equations. Secondly it is well accepted that male and female children grow differently; hence the gender based WHO growth charts. It has also

been independently reported that irrespective of HIV status, girls have a higher arm fat percentage than boys (32). In the case of HIV-infected children there is a hypothesis that the virus affects the body composition of the different genders differently (8). However, as a result of the relatively small sample size the study was likely inadequately powered to detect even moderate differences between sexes. Post hoc power calculations are however uninformative. The results of comparisons between the boy and girl groups need to be treated with caution.

3 RESULTS

3.1 Participants

Forty six participants signed informed consent for the parent study as seen in Figure 3-1. From this initial group six did not qualify for the parent study: two participants were not able to supply saliva samples (the samples were required according the parent protocol before the DXA scan was done), two children were no longer within the target age-band at the time of initiation of HAART as a result of delays, one child did not complete all study procedures within the 10 day window period as required in the protocol and the last child did not complete the required DXA scan. Two further participants' were unable to be included in the study as their caregivers withdrew consent during the screening, enrolment and baseline procedures. This meant that a total of 37 children took part in the parent study.

Number of participant who signed the consent form		n=46
	Number of participants that did not qualify for the study	n=6
	<ul style="list-style-type: none"> • Could not give saliva sample for the parent study 	n=2
	<ul style="list-style-type: none"> • Inappropriate age for study 	n=2
	<ul style="list-style-type: none"> <ul style="list-style-type: none"> • Needed HAART before 9 month of age 	n=1
	<ul style="list-style-type: none"> <ul style="list-style-type: none"> • Change in protocol not approved before child needed HAART 	n=1
	<ul style="list-style-type: none"> • Baseline data not collected within the 10 day window period after starting HAART 	n=1
	<ul style="list-style-type: none"> • Did not complete DXA scan 	n=1
	Lost to follow up	n=1
	Withdrew from the study	n=2
	<ul style="list-style-type: none"> • Mother started a new job and would not be able to attend monthly visits 	n=1
	<ul style="list-style-type: none"> • Mother withdrew on day of signing consent 	n=1
Number of participants included in the baseline analysis		n=37

Figure 3-1: Flow diagram of participant enrollment

3.1.1 Missing data

Some participants had missing data, particularly for protocol specified blood tests, which were not part of the routine standard of care and/or skinfold thickness measurements. With regards to the anthropometric results 32 children had the complete set of anthropometric measurements, one child did not have subscapular measurements and four did not have any of the required skinfold measurements. This resulted in 32 observations used for the Slaughter, Shaikh & Mahalanabis and Goran regression equations and 33 observations for Dezenberg regression equations. There was no evidence that children with missing data were systematically different to those without missing data.

3.1.2 Demographic and clinical and body composition characteristics

The demographic and clinical data of the study participant are tabulated in Table 3-1. Of the 37 participants that completed the study 19 (51%) were male and all were black South African children. The median ages of the children were 13 months (IQR 1 ; 20) and all were infected with HIV. Less than half (43%) had documented NVP exposure as part of PMTCT, 35% had no NVP exposure and 22% of the children's caregivers did not know if NVP was given to the child. Immunization was up to date in 91% of the children according to road to health cards. More than half of the children (60%) had delayed milestones. All of the participants met the criteria for initiation of HAART as per protocol, 46% and 54% had been classified WHO stage 3 and 4 respectively. The majority (89%) of children were on TB treatment, partly as a result of participant selection processes.

The median CD4 percentage was 14% (IQR 11; 20) and the HIV-1 RNA viral load was 140 000 (IQR 35 000 ; 500 000). The median haemoglobin (Hb) was 10 mg/dL (IQR 9 ; 11). The boys had a statistically significantly lower Hb than the girls. The remainder of the blood results were predominantly within normal ranges with no apparent difference by sex.

The median WAZ score was -2,6 (IQR -3,6 ; -1,2). While the boys tended to have lower WAZ compared to the girls there was no significant statistical difference between the two sexes. The median HAZ score was -2,7 (IQR -4,1 ; -1,6). The WHZ score median was -1,3 (IQR -2,3 ; -0,3) with some evidence $p=0,043$ that the male and female groups were significantly different with the median WHZ of the boys lower -2,1 (IQR -3,4 ; -0,4) than the girls -1,1 (-1,7 ; 0,1). The median MUAC was 13,5 cm (IQR 12,5 ; 14,7). There was no significant differences in the two groups with regard to triceps skinfold z-score and subscapular z-score.

DXA scans were conducted on all 37 participants. There was no significant difference in BF% between the genders. The median BF% (without head) as determined by DXA was 25% (IQR 22 ; 32). The sub-total FM (without head) was 1 590g (IQR 1 200 ; 2 420). The amount of FM in the legs, was the highest of the three regions of the body, then trunk fat and arm fat recorded the lowest of the three regions with only 13g (IQR 1 ; 40). The trunk was ranked as the most FFM of the three regions, recorded as 2700g (IQR 2030 ; 2960). The FFM for the arms was the least of the three regions with 630g (IQR 400 ; 750).

Table 3-1: Demographics and clinical characteristics of participants

Characteristics	N	Total	Male	Female	P value
Sex	37	N/A	19 (51%)	18(49%)	N/A
Age in months (IQR)	37	13 (11;20)	13 (10;20)	14,5 (12;20)	0.23
Weight (Kg) (IQR)	37	8.2 (6,5 ; 9,3)	7.4 (6.0 ; 8.9)	8.1 (6.4 ; 9.18)	0.36
WHO stage					
Stage 3 n (%)	37	17 (46%)	9 (47%)	8 (44%)	0.56
Stage 4 n (%)		20 (54%)	10 (53%)	10 (56%)	
TB at start of ART n (%)	37	33 (89%)	18 (55%)	15 (46%)	0.34
PMTCT exposed (%)	36	16 (43%)	9 (47%)	7 (39%)	0.695
Immunisations up-to-date (%)	32	29(91%)	15 (88%)	14 (93%)	0.763
Developmental milestones met (%)	37	15 (41%)	7 (37%)	8 (44%)	0.638
CD4%, median (IQR)	37	14% (11; 20%)	13% (10 ; 21)	15% (13 ; 20)	0.553
CD4+ cell count, median (IQR)	37	874 (447 ; 1236)	695 (407 ; 1304)	786 (509 ; 1236)	0.543
Viral Load, (copies/ml) median (IQR)	37	140 000 (35000 ; 500 000)	140 000 (33 000 ; 560 000)	146 500 (39 000 ; 440 000)	0.951
Hemoglobin (Hb), median (IQR)	36	10 (9 ; 11)	9,1(8,4 ; 10,3)	10,9 (9,6 ; 11,6)	0.0117
ALT (U/L), median (IQR)	35	23 (15 ; 33)	23 (13 ; 34)	21 (18; 3)	0.9873
Cholesterol (mmol/L), median (IQR)	34	2,5 (2,2 ; 3,2)	2,7 (2,1 ; 3,3)	2,5 (2,3 ; 3,2)	0.8946
Triglycerides (mmol/L), median (IQR)	32	1,9 (1,4 ; 2,4)	2 (1,6 ; 2,3)	1,85 (1,4 ; 2,4)	0.7318
Albumin (g/L), median (IQR)	30	36 (34 ; 40)	36,5 (32 ; 40)	36 (35 ; 38)	0.7932

WAZ, median (IQR)	37	-2,6 (-3,6 ; -1,2)	-3,1 (-4,5 ; -1,2)	-1,7 (-2,9 ; -1,2)	0,114
HAZ, median (IQR)	37	-2,7 (-4,1 ; -1,6)	-3,4 (-4,3 ; -1,9)	-1,9 (-3,4 ; -1,2)	0,136
WHZ, median (IQR)	37	-1,3 (-2,3 ; -0,3)	-2,1 (-3,4 ; -0,4)	-1,1 (-1,7 ; 0,1)	0,043
BMI, median (IQR)	37	-1,1 (-2,1 ; -0,1)	-1,8 (-2,7 ; -0,3)	-0,9 (-1,4 ; -0,1)	0,059
Head circumference Z-score, median (IQR)	35	-1 (-1,7 ; 0,1)	-1,2 (-2,5 ; -0,6)	-0,7 (-1,3 ; 0,1)	0,186
MUAC Z-score, median (IQR)	33	-0,8 (-1,73 ; -0,3)	-0,8 (-3,9 ; -0,03)	-0,8 (-1,6 ; -0,4)	0,705
MUAC cm, median (IQR)	33	13,5 (12,5 ; 14,7)	13,2 (11,1 ; 14,7)	13,5 (12,6 ; 14,5)	0,9713
Triceps SFT Z-score, median (IQR)	33	-1,2 (-2,2 ; -0,1)	-1,6 (-2,2 ; 0,2)	-0,9 (-1,8 ; -0,4)	0,349
Subscapular SFT Z-score, median (IQR)	34	-1,2 (-2,5 ; -0,1)	-1,7 (-2,7 ; 0,4)	-1,0 (-2,4 ; -0,2)	0,580
BF% (without head) :DXA, median (IQR)	37	25% (22 ; 32)	25% (20 ; 32)	27 (23 ; 32)	0,27
BF% (with head) DXA, median (IQR)	37	24% (22; 29)	23% (21 ; 29)	25% (22 ; 29)	0,41
FM (without head) DXA (g), median (IQR)	37	1591 (1205 ; 2419)	1451 (991 ; 2360)	1659 (1414 ; 2437)	0,26
Arm fat (g), DXA, median (IQR)	37	13 (1,2 ; 42,1)	15 (1,8 ; 81)	10 (0,8 ; 42)	0,41
Leg fat (g), DXA, median (IQR)	37	810 (560 ; 1220)	720 (410 ; 1350)	1030 (700 ; 1220)	0,15
Extremity fat DXA (g), median (IQR)	37	1470 (1200 ; 1920)	1460 (1150 ; 1840)	1520 (1200 ; 1930)	0,17
Trunk fat (g)DXA, median (IQR)	37	689 (520 ; 930)	700 (500 ; 930)	630 (570 ; 950)	0,69
Arm lean (g) DXA, median (IQR)	37	630 (400 ; 750)	670 (350 ; 790)	621 (440 ; 730)	0,86
Leg lean (g) DXA, median (IQR)	37	860 (760 ; 1190)	860 (760 ; 1100)	870 (760 ; 1220)	0,68
Trunk lean (g) DXA, median (IQR)	37	2700 (2030 ; 2960)	2620 (2030 ; 2960)	2800 (2030 ; 3100)	0,38

Two sample Wilcoxon rank-sum (Mann-Whitney) test

Table 3-2 tabulates the distribution of the children's FM in the body. The proportion of the total body fat mass which was located in the arms was 0,7% (IQR 1,1 ; 4,5) was the smallest

of the three regions, while the most of the body fat mass was located was in the legs 54% (IQR 49; 62). The extremity regions recorded a total of 56% (IQR 51 ; 63) of sub-total body fat mass with the remainder located in the trunk. The trunk to extremity fat ratio was 0,8 (IQR 0,6 ; 1,0).

Table 3-2: DXA scan result: Distribution of fat mass in the body

Region of the body (without head)	N	Median(IQR)	Male	Female
% FM in Arms	37	0,7% (1,1 ; 4,5)	0,74% (0,2 ; 6)	0,48% (0,04 ; 2)
% FM in Legs	37	54% (49 ; 62)	53% (46 ; 62)	55% (50 ; 63)
% FM in Trunk	37	44% (37,1 ; 50)	45% (38 ; 54)	43% (36 ; 48)
Extremity fat %	37	56% (51 ; 63)	55% (46 ; 62)	57 (52 ; 64)
Trunk to extremity ratio	37	0,8 (0,6 ; 1,0)	0.8 (0.6 ; 1.2)	0.7 (0.6 ; 0.9)

3.1.2.1 BF% and FM (g) results from the regression equations and DXA scans

The BF% results from DXA and the regression equations that predict BF% Slaughter (1984) and Shaikh & Mahalanabis (2004) are summarised in Table 3-3. The lowest BF% of 10% (IQR 9 ; 13) was obtained from the Slaughter (1984) equation and the highest from the DXA scans at 26% (IQR 22 ; 32). The limits of agreement for both equations were approximately 15%.

Table 3-3: BF% results from DXA scan (without head) and regression equations

Measurement method	N	% Fat Mass, median (IQR)	Bias (SD) (DXA-equation)	Limits of agreement (Regression-equation)	Lin Correlation Coefficient	Pearson correlation Coefficient
DXA (without head)	37	25% (22 ; 32)	N/A	N/A	N/A	N/A
Slaughter (1984)	32	10% (9 ; 13)	15% (3.7)	8,0 ; 23%	0,07	0.65
Shaikh & Mahalanabis (2004)	32	19% (18 ; 20)	8% (3.9)	-0,1 ; 15 %	0,16	0.629

3.1.2.2 Scatterplots for Slaughter (1984) and Shaikh & Mahalanabis (2004) regression equations compared to DXA results

Slaughter (1984) regression equation

The scatter plot (Figure 3-2.a) of the Slaughter (1984) regression equation and DXA results demonstrated a positive correlation between the results, with some suggestion that the association maybe linear. The Slaughter equation results however constantly underestimated the BF% of the children compared to DXA.

Shaikh & Mahalanabis (2004) regression equation

The scatter plot (Figure 3-2.b) of the Shaikh & Mahalanabis (2004) equation and DXA results also demonstrated a positive correlation. The regression equation however consistently underestimated the BF% of the children. There was some suggestion that the

relationship between the Shaikh & Mahalanabis equation and DXA measurements was non-linear, particularly at higher values of DXA BF%. This was displayed by the higher regression BF% results which registered between 16% and 22% while the DXA results ranged from 20% to 37%.

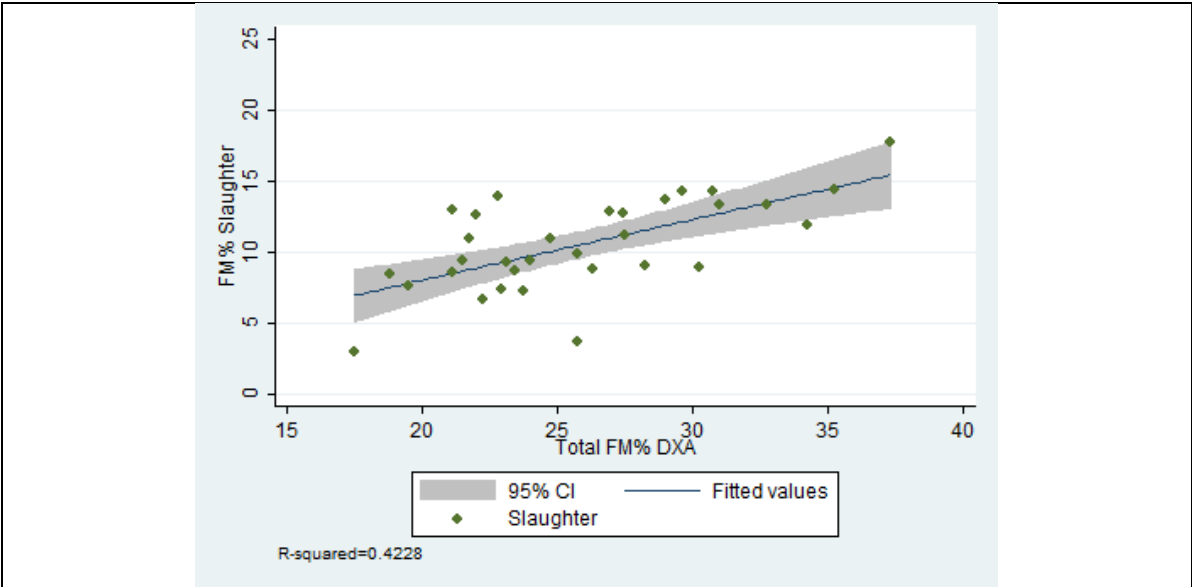


Figure 3-2.a: Slaughter regression equation and FM% calculated from DXA

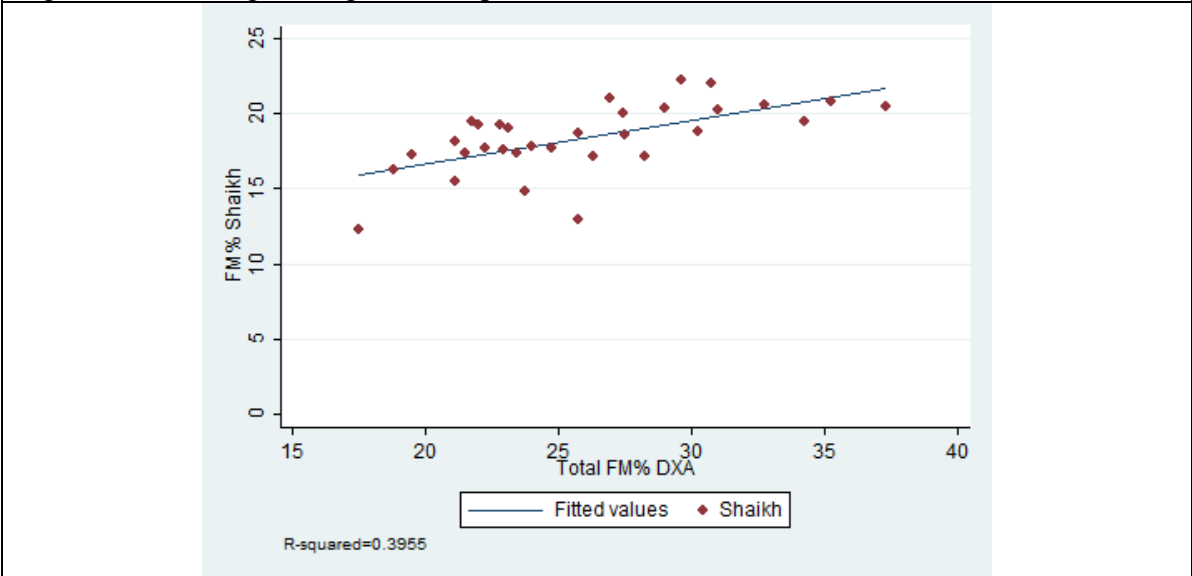


Figure 3-2.b: Shaikh & Mahalanabis regression equation and BF% calculated from DXA

Figure 3-2: Scatter plots of Slaughter and Shaikh & Mahalanabis regression equations and BF% calculated from DXA

3.1.2.3 Bland-Altman plots for Slaughter (1984) and Shaikh & Mahalanabis (2004)

regression equations and DXA results

Slaughter (1984) regression equation

In the Bland-Altman plot (Figure 3-3.a) of the Slaughter (1984) equation and the DXA results, the scattering of results indicated that as the mean BF% increased so did the difference between the two methods. Consequently, the Slaughter equation displayed less agreement with the DXA results as the BF% increased. Furthermore, the wide range of limits of agreement of 8,0% to 22,7% with an average difference of 15.4% SD (3,7) add to the conclusion that there is poor agreement between the methods. This equation displayed the weakest agreement of the four equations as indicated by the Lin Concordance Correlation Coefficient of 0.07.

Shaikh & Mahalanabis (2004) regression equation

The Bland-Altman plot (Figure 3-3.b.) of the Shaikh & Mahalanabis (2004) regression equation showed a scattering of results that indicate, as the mean BF% of the two methods increased so did the difference in the measurements. This outcome indicated that the regression equation had better agreement at lower BF% values. The agreement between DXA and this regression equation is weak as the limits of agreement are wide (-0,1% to 15,2%) and show an average difference of 7,5% SD (3,9).

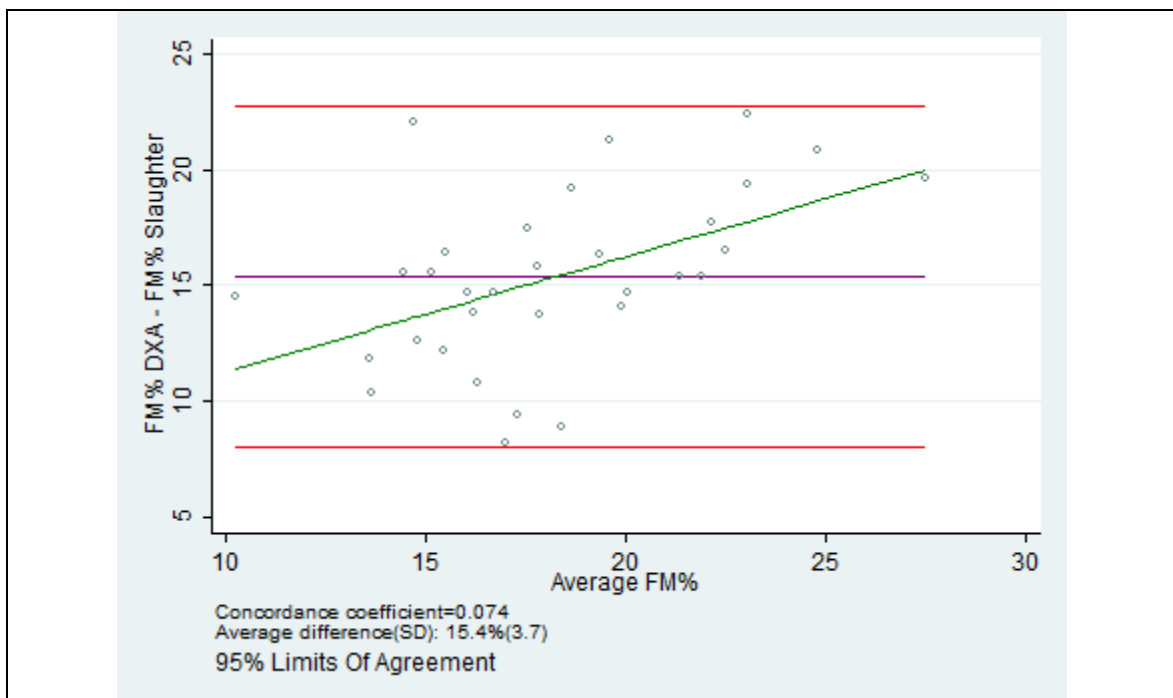


Figure 3-3.a: Comparing BF% measured by Slaughter's regression equation (1984) and DXA

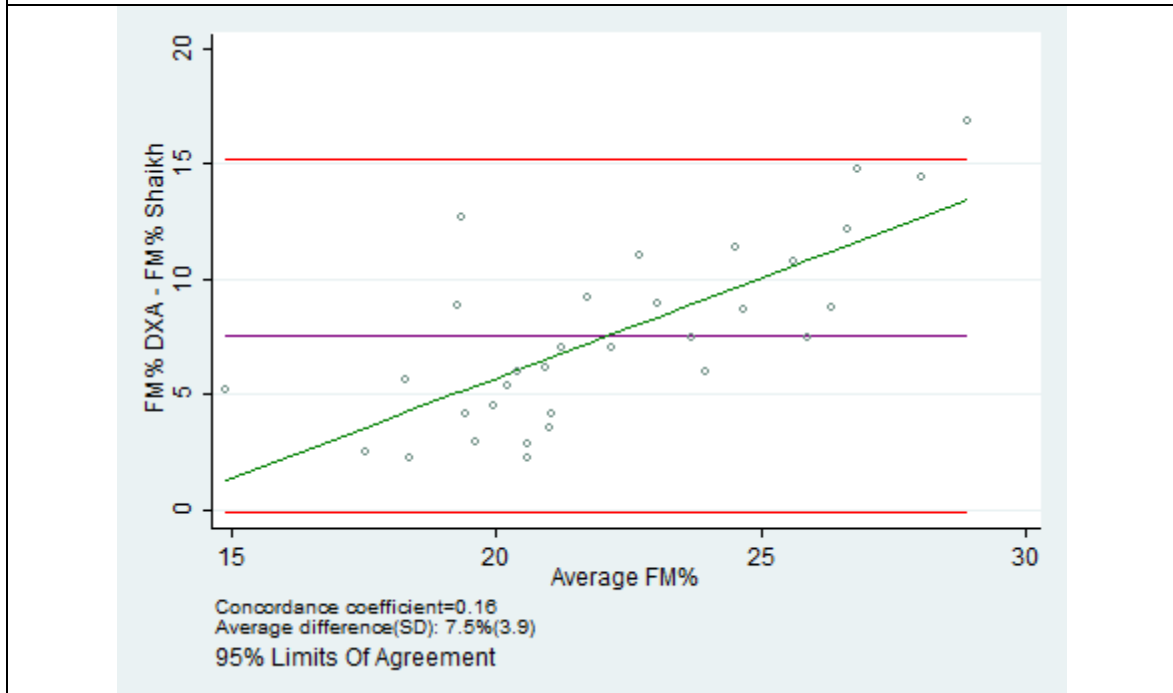


Figure 3-3.b: Comparing BF% measured by Shaikh & Mahalanabis's regression equations (2004) and DXA

Figure 3-3: Bland-Altman plots comparing %BF as predicted by Slaughter and Shaikh & Mahalanabis's to DXA

The FM (g) results from DXA and the regression equations that predict FM (g), Dezenberg (1999) and Goran (1996) are presented in

Table 3-4. The Dezenberg equation resulted in a negative predicted median FM (g) of -1823g (IQR -2540 ; -1160). Although the median FM (g) predicted by the Goran equation is positive at 296g (IQR -83 ; 1116), this was still lower than the DXA results of 1952g (IQR 1594 ; 2847)

Table 3-4: FM results from DXA scan (without head) and regression equations

Measurement method	N	Total Fat (g), median (IQR)	Bias (SD) (DXA-equation)	Limits of agreement (Regression-equation)	Lin Correlation Coefficient	Pearson correlation coefficient
DXA (with head)	37	1952g (1594 ; 2847)	N/A	N/A	N/A	N/A
Dezenberg (1999)	33	-1823g (-2450 ; -1160)	3549 (661)	-2252 ; 4844	0,1	0.83
Goran (1996)	32	296g (-83 ; 1116)	1311 (401)	523 ; 2098	0,35	0.87

3.1.2.4 Scatterplots for Dezenberg (1999) and Goran (1996) regression equations compared to DXA results

Dezenberg (1999) regression equation

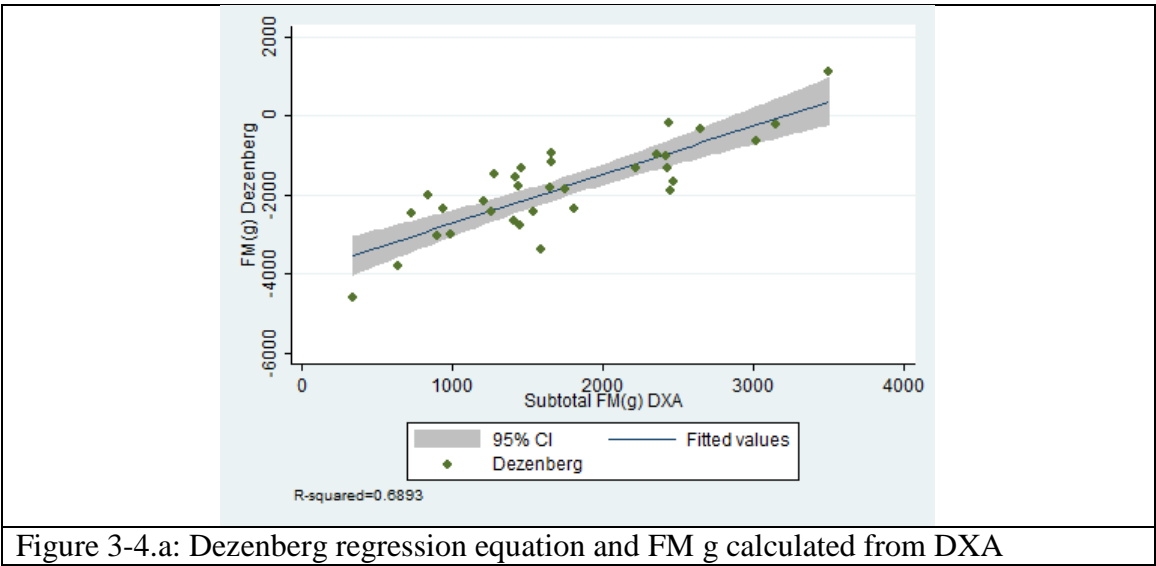
The scatter plot (

Table 3-4.a) regression equation and the DXA results showed a positive correlation, with a strong suggestion of linearity. The regression equation constantly underestimated the FM (g) of the children. As the FM (g) of the children increase the regression equation results had better agreement. All except one result had a negative FM.

Goran (1996) regression equation

The scatter plot (

Table 3-4.b) Goran (1996) regression equation and the DXA results showed a positive correlation. The regression equation consistently underestimated the FM (g) of the children and ten of the results were negative.



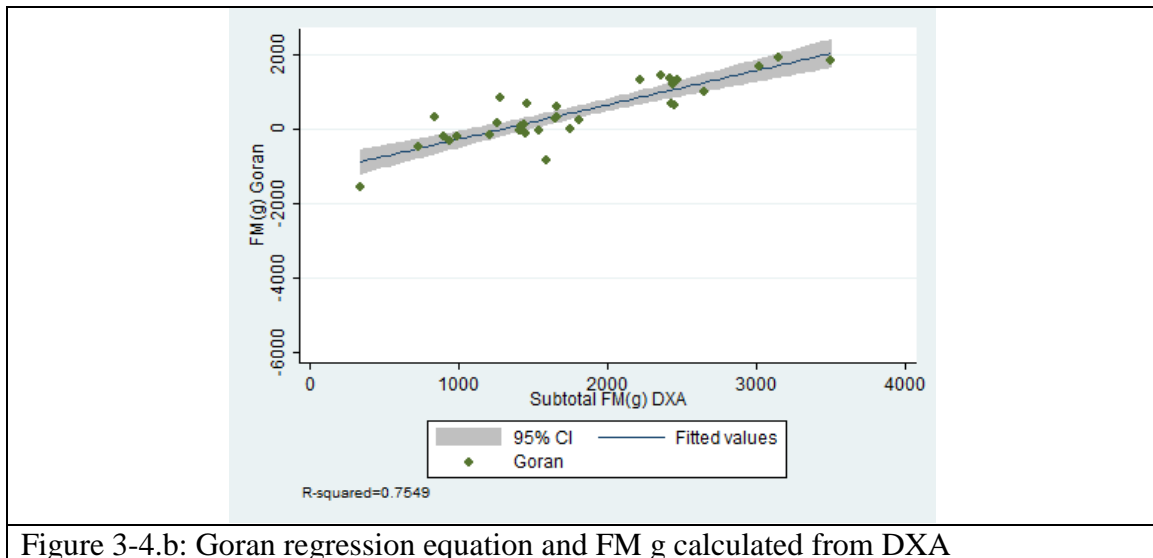


Figure 3-4: Scatter plots of Dezenberg and Goran regression equations and FM (g) calculated from DXA

3.1.2.5 *Bland-Altman plots for Dezenberg (1999) and Goran (1996) regression equations and DXA results*

Dezenberg (1999) regression equation

The Bland-Altman plot (Figure 3-5.a) between Dezenberg (1999) regression equation and the DXA results showed a scattering of results that, as the mean FM (g) difference of the two methods increased, so the difference between the two measures decreased. This showed that as the FM (g) of the child increases so does the agreement of the two methods. However, the limits of agreement are still wide and range from -2252g to 4844g with an average difference of 3548,6g SD (661). The Dezenberg equation had the second weakest agreement to DXA as shown by the Lin Correlation Co efficient of 0,1.

Goran (1996) regression equation

The Bland-Altman plot (Figure 3-5.b) for the Goran (1996) regression equation and the DXA results showed a scattering of results from approximately 500g to 2000g. This indicated a

near constant error that showed a slight decrease as the FM (g) increased. However, the two methods do not indicate good agreement as the limits of agreement were 523g to 2099g with an average difference of 1311,1g (401,9). The Goran (1996) equation achieved the best agreement of all four equations as shown by the Lin Concordance Correlation Coefficient of 0,35.

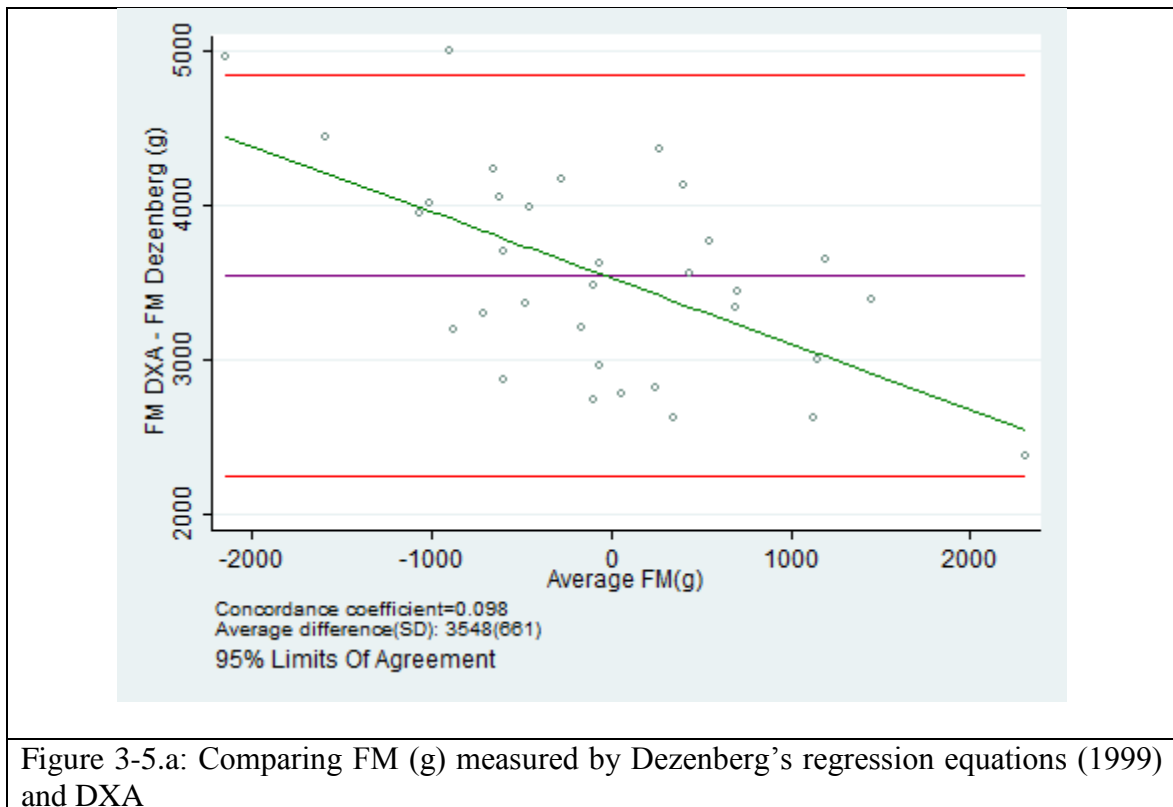
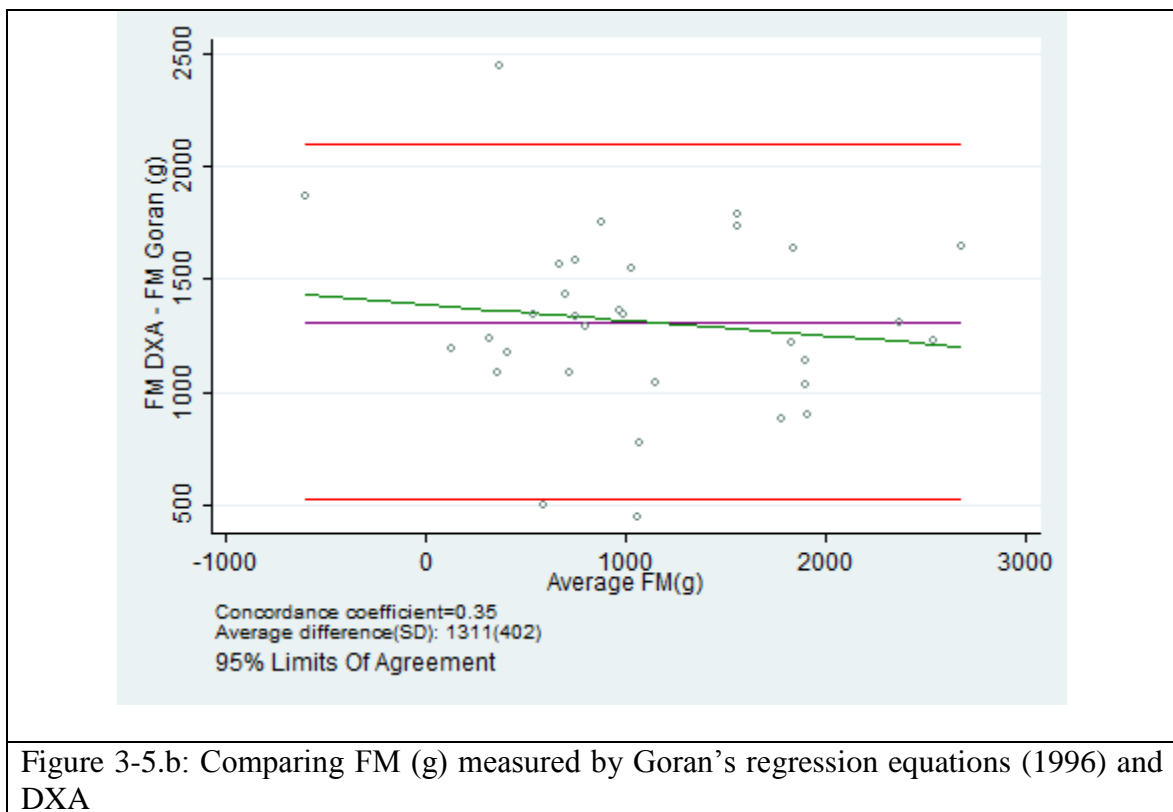


Figure 3-5.a: Comparing FM (g) measured by Dezenberg's regression equations (1999) and DXA



3.2 Relationship between BMI and BF%

The scatter plot for BF% vs. BMI shows that as the BMI of a child increases so does the BF% as shown in Figure 3-6.

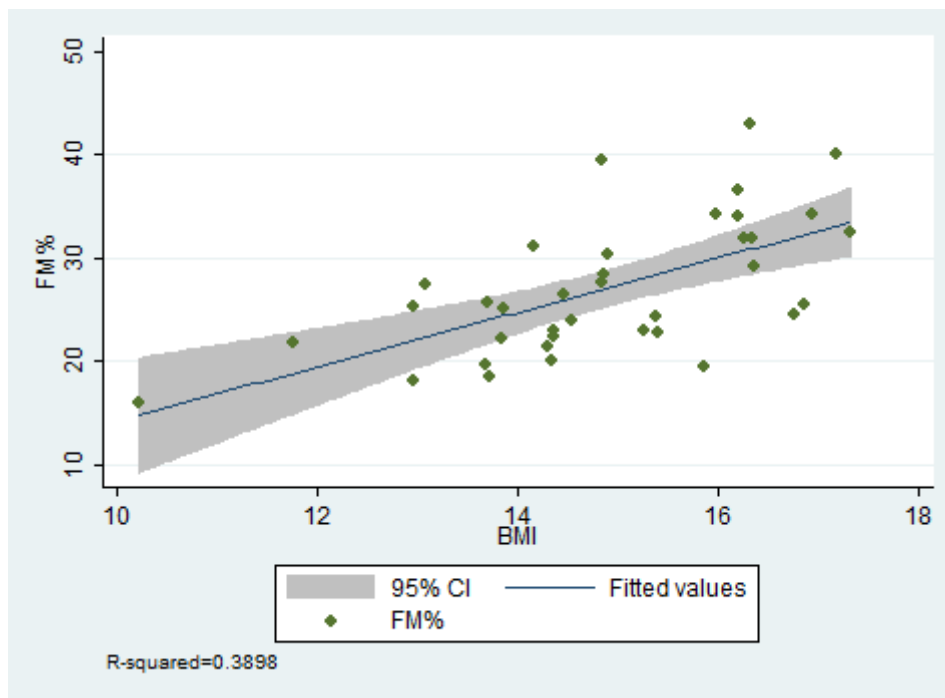


Figure 3-6: Scatter plot of BF% versus BMI

3.3 Analysis of measurement reliability

To assess the reliability of the measurements taken, the percentage variability between repeat measurements were calculated by subtracting the second measurement from the first and then dividing by the first. The inter coefficient of variation of the study dietician was determined to be acceptable. This was ascertained by comparing the results taken by the study dietician from the same child. The reliability of the basic anthropometric parameters was acceptable. The most reliable of the anthropometric parameters was weight where only one pair (2.7%) of the observations had a percentage difference of >1%. The least accurate was that of triceps where 18% of the measurements taken had a difference greater than 5%.

The mean variance of the triceps and subscapular skinfold measurements were similar at 2,9% and 3,0% respectively. The tricep and subscapular measurements were considered reliable as they were well within the recommended range of > 66% of paired measurements to be within 5% difference of each other (43). Table 3-5 tabulates the mean variance and the percentage of observations within the required range of percentage difference (<1% for weight, height, head circumference and MUAC and <5% for tricep and subscapular skinfolds)

:

A substitute dietician took 5 sets of the anthropometric results. This dietician was trained and her technique was observed by the study dietician. However their intra coefficient of variation was not tested. As they did not measure the same child at same child at the same visit.

Table 3-5: Assessment of reliability of anthropometric measurements

Measurement	Mean variance	% of observations outside of acceptable difference limits
Weight (kg)	0,0009	2,7
Height (cm)	0,0035	8,1
Head Circumference (cm)	0,0041	10,8
MUAC (cm)	-0,002	15,2
Triceps (mm)	2,9	18,8
Subscapular (mm)	3,0	17,6

In Figure 3-7 Bland Altman plot for scale weight 1 vs. scale weight 2, the agreement between the two scale weights closely scattered around the 0 with the limits of agreement of -40g and 40g. Thus the two measurements taken of the same child were very closely in agreement as

can be seen by the mean variance of 0,0009 and 2,7% of all observation within 1% of each other.

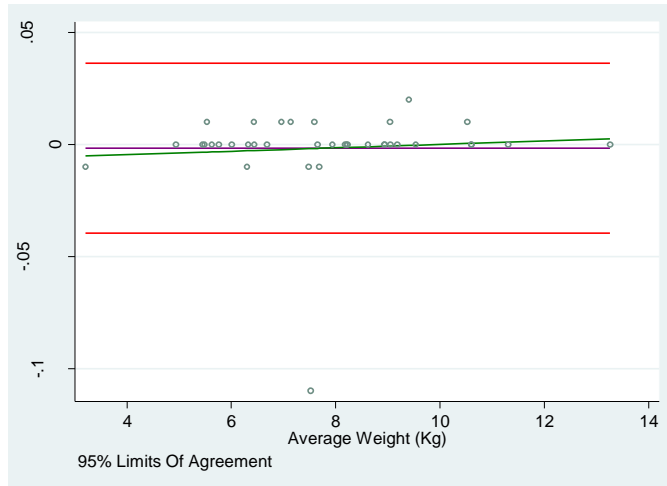


Figure 3-7: Bland Altman plot for scale weight versus scale weight

When the DXA total weight and the weight measured with the scale are compared in Figure 3-8, the results correlate closely with a small mean difference of 285g (range from -500g to 1.16kg). On average the DXA showed a total mass of 285g heavier than the scale. It is important to note that in some cases the DXA and scale weight were not measured on the same day.

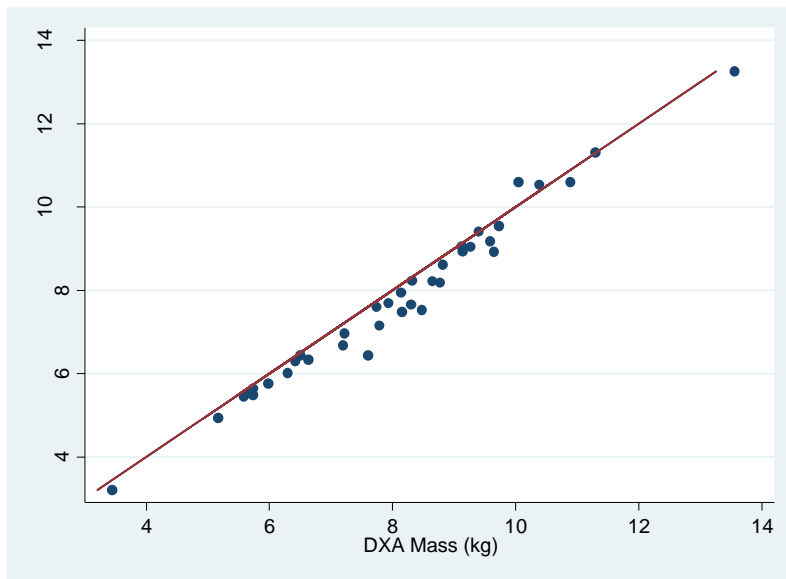


Figure 3-8: DXA weight versus average scale weight

The CV for the DXA technician was <1% for scanning and analyzing. The phantom results for the DXA machine have a CV of 0.439% for Area cm^3 , 0.917% for BMC (g) and 0.75% for BMD (g/cm^3). The figures are attached in Appendix I.

4 DISCUSSION

There are no studies which have reported DXA results of body composition in children aged 9-36 months who are infected with HIV. This study is the first to report on the fat mass, fat free mass and body fat percentage of young black South African children who are infected with HIV prior to the commencement of HAART. As there are no reference data for the body composition of healthy South African children in this age group, it is not appropriate to draw firm conclusions on the effect of HIV, or HIV and TB, on body composition from the results of this study.

A few studies have described the body composition of HIV-infected American children. The results from these American based studies have been included in the discussion to assist with interpreting the current results. However it needs to be stressed that the results of the American studies were obtained from children of different ethnic groups living in a developed country. Furthermore the age groups of the children studied are not identical and some of the American studies were of children who had been on HAART which is known to have an effect on body composition especially regimens that include protease inhibitors (29).

The discussion focuses predominantly on the descriptive data, comparing these with the findings from other studies, and the outcomes of the Bland-Altman comparison of agreement between the four regression equations and the DXA scan results.

4.1 Body fat % results from DXA

The BF% (without head) of the children was 25% (IQR 22 ; 32), male 25% (IQR 20 to 32) and female 27% (23 ; 32). When the head was included in the assessment, the BF% result was 24% (IQR 22 ; 29) male and female 25% (IQR 22 ; 29).

Jacobson (29) published a study that compared the body composition of 7-16 year old HIV-infected and HIV-uninfected children from 15 American cities. The HIV positive children, who were on HAART, had a lower total BF% (including head), 18% for males and 25% for females, than the HIV-uninfected children. In comparison to the data of this study, the Jacobson's study revealed that the female HIV-infected children population had similar BF%, despite them being older and already on ARV treatment. In contrast, the HIV-infected male population in the Jacobson's group exhibited less BF% than the males in this study.

4.1.1 Interpretation of DXA BF% results

The interpretation of the DXA results conducted on the children in this study is very difficult and should be read with caution. There is no control group or comparable reference group to which this study can be compared with.

However there are three studies that have given BF% cut offs that may give some insight to the relevance of this study's results. Reilly (25) used BF% for his classification of under malnutrition (<5% males and <12% females) of all children under the age of 18 years.

Therefore if Reilly's classification for under nutrition is used none of the children in this study can be classified as under nourished when using their BF%. Taylor's classification for overweight and obesity has BF% cut offs at the BMI (kg/m^2) cut offs for overweight (BMI 25) for male BF% 20 (19:21) and female 21 (19,21) and obese (BMI 30) for male BF% 26 (25,29) and female 26 (25,27). When Taylor's cutoffs are applied to this study group, 14 children are classified as overweight while 17 children are classified as obese. Higgins (20) cut off ($>33\%$ BF) for a 15 times more likely risk of having increased lipid and blood pressure profiles would apply to 7 of our children. The limitation of the above mentioned cut offs is that they were developed in older children (2 years and up) and should be used with caution.

By using %BF as part of the nutritional assessment, a more complex picture is developed. As the common nutritional description of children that are infected with HIV and in need of treatment are children that have growth failure and who are stunted and/ or underweight (8). The addition of BF% shows that these children may show signs of underweight and an increased risk of lipid and blood pressure problem. A possible reason for this study population's high BF% could be a result of the HIV infection, where HIV-infected children lose predominantly FFM and maintaining FM thus resulting in a relatively high BF% despite being undernourished. If the weight loss experienced by the HIV-infected children is predominantly FFM, this has an effect on the severity of their disease, an increased HIV viral load, mortality, a decrease in quality of their life's, reduced immune function, reduced tolerance of ART therapy and an increased cost of medical care (6, 17).

4.2 Distribution of fat mass

4.2.1 Region of the body with the highest BF%

The distribution of FM in the body is of clinical importance in both HIV-infected and uninfected populations with parameters such as waist circumference associated with an increased insulin and insulin to glucose ratio in HIV positive adults (69). In this study most of the FM 54% (IQR 49 ; 62) was situated in the legs, with 44% (IQR 37,1 : 50) in the trunk and with less than 1% located in the arms 0,7% (IQR 1.1 ; 4,5).

Arpadi (32), published an analysis of the body composition and fat distribution in HIV-infected (n=64) and uninfected, healthy 6-16 year old children (n=157). The data was obtained from two other studies that had been previously conducted in New York City, USA. Ninety four percent (94%) of the HIV-infected children were on HAART (32). While the HIV-infected and uninfected children had similar BF%, the percentage of fat located in the arms was significantly different, with HIV-infected children having a higher percentage of fat in the arms. In the findings of Arpadi (32) 44% of FM of HIV-infected children was located in the children's legs, 32% in the trunk and 13% in the arms. The percentage of fat mass located in the arms in our study was much lower at 0,7% (1,1 to 4,5). The reason for the difference in percentage of FM located in the arms is unclear. However, one possibility is that this is related to HAART as most children in the Arpadi study were already on HAART at the time of the study, and there was evidence in this study that the arm fat increased over time (32).

4.2.2 Trunk to extremity fat ratio

The trunk to extremity fat mass ratio is an important measurement as is a measure of abdominal obesity (70). The children of this study had a trunk to extremity fat ratio of 0,8 (IQR 0,6 to 1,0), which indicated more FM in the extremities than in the trunk. These results are similar to those published by Jacobson (29). which showed a trunk to extremity fat ratio of 0.83 (CI 0,78 ; 0,85) for males and 0,84 for females (CI 0,0,8 ; 0,89) (29). Unfortunately there are no published reference ranges for trunk to extremity fat ratio in young children making it difficult to interpret these results further.

4.2.3 Comparison of DXA results to Shypailo's et al (2008) published results

Comparison of DXA results with those published by Shypailo et al. As noted earlier Shypailo (53) conducted a study of the reliability of DXA scans in children when using two different software versions. Their publication provides data on FM and BF% in a small group of children 1- 3 years of age. When the DXA results for BF% and total FM were compared to those of Shypailo et al., the absolute FM (g) values obtained in our study population were significantly lower ($p=0.0001$) for both males and females (Table 4-1). Comparison of study results to data from Shypailo et al. were performed using the immediate Student's t-test for comparison of two means in Stata which incorporates the mean, standard deviation and sample size for each of the two independent samples.

There is also some evidence ($p=0,027$) that the means of BF% for females were different in the two studies, with our study population having a lower BF%. The mean BF% of the boys

was however similar. While the lower total body fat (g) found in our study is likely to be at least partly related to the HIV-positive status, interpretation of the similar BF% in boys and difference BF% in girls is complicated by a number of limitations arising from the indirect nature of the comparison between the two study groups. These limitations include the country of origin, socio-economic status, ethnicity and software versions used (12.1 vs. 12.5). In addition the children in this study were nine to thirty six months compared to one to three years in the Shypailo study. Sample sizes in both studies were also relatively small. Despite these limitations it is interesting that a different pattern was detected in the BF% of the male and females. This observation may indicate that the sexes are affected differently by HIV infection. Arpadi (8) found that in boys with normal growth and HIV-infection, that there was a decrease in FFM, however this was not found in the girls with normal growth and HIV infection (8).

Table 4-1: Comparison of study results to data from Shypailo et al (2008)

Data	Gender	Shypailo et al. (2008) version 12.1 (53)	DXA Scan results, mean (SD)	P value
BF%	Male	24.9% \pm 3.4	25,9 \pm 6.6	0.72
	Female	34.4% \pm 7.5	28.2 \pm 6.5	0.027
FM (g) total	Male	3260 \pm 500	1583 \pm 731	0.0001
	Female	4360 \pm 800	1835 \pm 780	0.0001

4.3 Agreement between regression equations and DXA

This study shows that none of the four regression equations have reasonable agreement with DXA scans in HIV-infected South African children aged nine to thirty six months, many of whom were co-treated for TB. Reasonable Pearson correlation coefficients of 0.87 and 0.83

were obtained using simple linear regression between DXA and the Goran and Dezenberg regression equations respectively. The Slaughter and Shaikh equations demonstrated lower correlations at 0.65 and 0.629, respectively. However, high correlation does not necessarily imply good agreement between the two methods. The results of the Bland-Altman analysis showed very high mean biases and wide limits of agreement with DXA scans for all four regression equations. The outcomes of the analysis of agreement for each of the four regression equations are discussed below in more detail.

4.3.1 The Slaughter regression equation (1984)

The Slaughter regression equation (1984) (

Table 4-2) was derived using a combination of U_{UW}, deuterium oxide dilution and photon absorptiometry in 310 children aged 8-29 years (51). Despite the equation having been developed in older children it is often used in routine practice by dietitians to assess BF% in young children in South Africa.

The scatterplots of the DXA and Slaughter regression equation showed that the regression equation underestimated the BF% by a large amount. The BF% obtained from the Slaughter equation remained within a fairly narrow range of 10-15% compared to the DXA range of 16 to 43%. This is also evident from the Bland-Altman plots, where it is apparent that as the mean BF% increased so the difference between the two methods increased. There was very

little agreement between the two methods shown by the mean bias of 15% (SD 3,7) and wide limits of agreement of 8% ; 22,7%.

It is not surprising that the Slaughter (1984) regression equation had poor agreement with DXA as the equation was developed for older children. Extending the use of regression equations to age groups which were not included in their development is unlikely to be useful. In addition to the age-group issue though, the equation incorporates the tricep and subscapular measurements to predict BF% and the DXA results show that in HIV-infected 9-36 month old children prior to the initiation of HAART that the majority of the FM is found in the legs with very little in the arms. This suggests that including an alternative anthropometric parameter such as the front thigh for the leg region or the abdominal region for the trunk might provide better agreement with BF% in this population. Secondly the development of the regression equation experienced some methodological problems as incorrect tanner staging (only one category of tanner was tested for staging, while the age group studied was 8-25 years) and the ethnicity of participants was poorly described (48).

It is however surprising that the Slaughter regression equation (1984), which is recommended for use in the study population (48), had the lowest agreement of all four equations. The recommendation to use this equation in this population was also challenged by Cameron (48) who also found poor agreement with DXA scan results in black South African children 9 years of age. The results of this study show that the Slaughter (1984)

regression equations are not compatible in our black South African HIV and TB infected study, population aged 9-36 months.

Table 4-2: Slaughter regression equation

Equation name	Equation	Population Tested	Derived from	Age group of participants
Slaughter (1984) (51)	Boys: $BF\% = 1.21 \times (T + S) - 0.008 \times (T + S)^2 - 3.2$	For black males (Tanner 1)	Body density from underwater weighing, photon absorptiometry & TBW derived from deuterium BF%	8-25 year olds but is used in younger children
	Girls: $BF\% = 1.33 \times (T + S) - 0.013 \times (T + S)^2 - 2.5$	For all females		

Key: T= triceps skinfold thicknesses (mm), S= Subscapular skinfold thicknesses (mm),

4.3.2 Shaikh & Mahalanabis regression equation (2004)

The Shaikh & Mahalanabis (2004) (Table 4-3) regression equation was derived from BIA and TBW for preschool, low socioeconomic Indian children (100 male and 84 female) (52). During development of this equation the authors examined a variety of skinfold sites and circumferences including bicep, tricep, subscap, suprailiac and MUAC. The authors found that in the undernourished [WAZ boys -1,893 SD (± 0.078) girls -2,097 SD (± 0.091), HAZ boys -1,064 SD (± 0.105) girls -1,438 SD (± 0.096) and WHZ boys -1,543 SD (± 0.069) girls -1,438 SD (± 0.096)] population that the equation including subscap, tricep sites, MUAC and age provided the best agreement with D₂O dilution method to predict BF%. Height measurements were not included for assessment because the researches were of the opinion that measurements of skinfold were more reliable and easier to obtain than height. Despite

having good agreement with other regression equations and BIA (tested with 50 participants), the published equations were reported as preliminary as only seven males and five females were tested for agreement against D₂O which was regarded as a reference method for the study.

The scatter plots for the Shaikh & Mahalanabis (2004) regression equation and DXA showed that the regression equation considerably underestimated the BF% and remained fairly range bound with a range of 12 – 22%. The Bland-Altman plot of the results showed that as the FM% of the children increased, so did the difference in the measurement between the two methods. While this equation had marginally better agreement with the DXA compared to the Slaughter equation the high mean bias of 8% (SD 3,9) and wide limits of agreement of - 0,1% : 15%. The equation is not suitable for use in black South African children who are infected with HIV and co-treated with TB.

Similar to the Slaughter (1984) equation, the Shaikh & Mahalanabis (2004) equations for boys and girls incorporate predominantly arm measurements to predict BF%, which is not where the majority of FM is found in our study population. It is difficult to compare these results to the Miller (1993)(10) and Fontana (1999) (33) studies. Firstly the Miller (1993) study used arm anthropometrics to calculate FFM. The study's main finding was that HIV infected children had significantly less FFM. In the study it also mentions that there is no change in FM when the HIV infected and uninfected groups are compared to each other. As

there is no control group for our study it is impossible to determine if the amount and distribution of FM in our children's arms is normal or not.

However the Fontana (1999) study found that there was a decrease in FFM and FM that was related to the amount of total weight loss. Once again without a control population it is difficult to interpret if the low distribution of FM in the arm is the main cause for the under prediction of BF% when using the Slaughter (1984) equation, the Shaikh & Mahalanabis (2004) equations compared to DXA scan results

Table 4-3: Shaikh & Mahalanabis regression equation (2004)

Equation name	Equation	Population Tested	Derived from	Age group of participants
Shaikh & Mahalanabis (2004) (52)	Boys; %fat= $5.304 + 0.269 \times T + 0.50 \times S + 0.685 \times M - 0.063 \times A$	Preschool, low social economic Indian Children	BIA and skinfold measurements TBW from deuterium oxide BF%	Boys: 26.8 months with a SD of 14.8 months Girls:30.2 months with a SD of 15.82
	Girls; % fat= $7.017 - 0.053 \times T + 0.201 \times S + 0.765 \times M + 0.052 \times A$			

Key: T= triceps skinfold thicknesses (mm), S= Subscapular skinfold thicknesses (mm), M= mid arm circumference (cm), A= Age (months)

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5 Appendix I: Phantom results for DXA machine

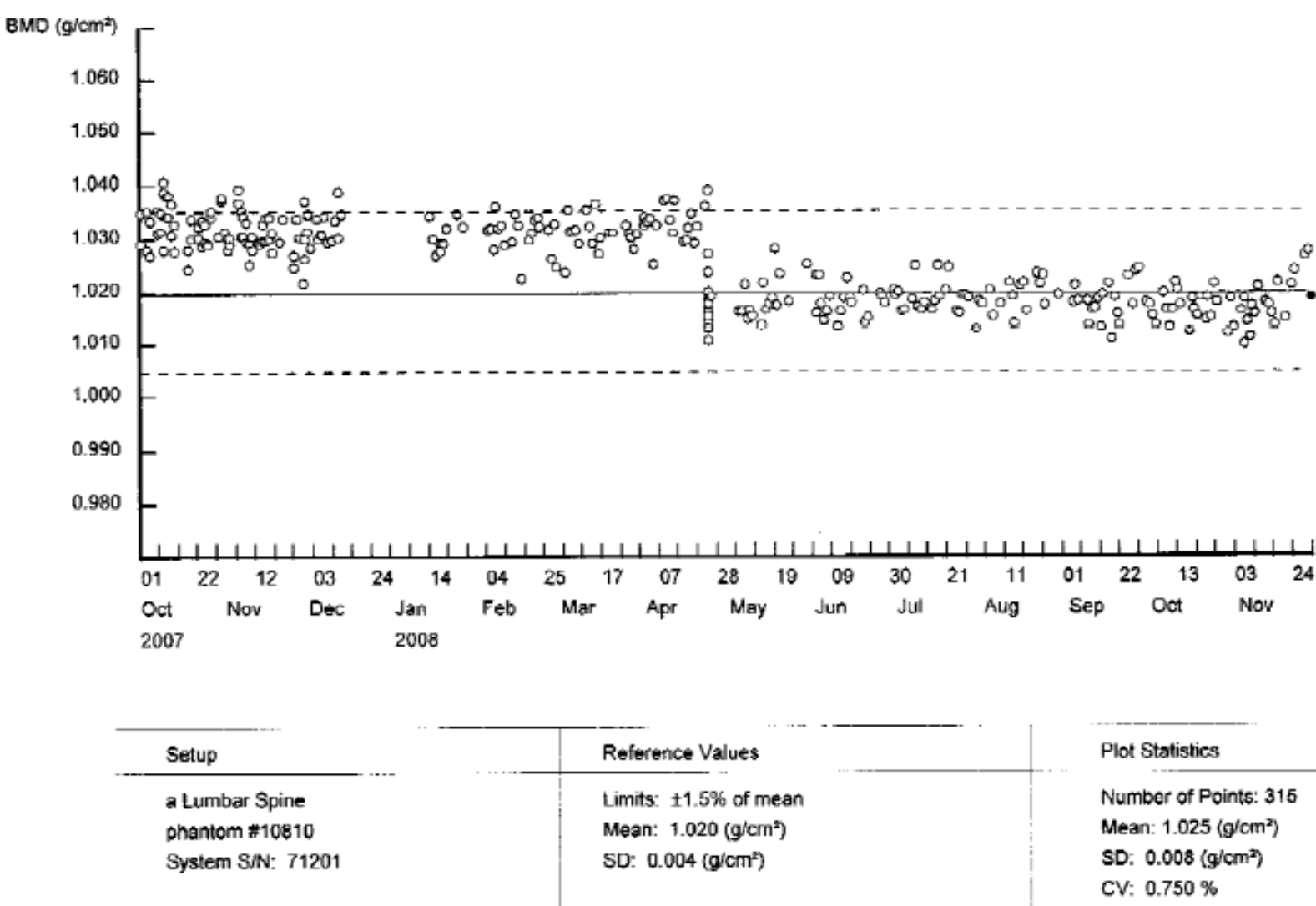


Figure 5-1: Phantom results for DXA machine form October 2007 till November 2008

BMD (g/cm²)

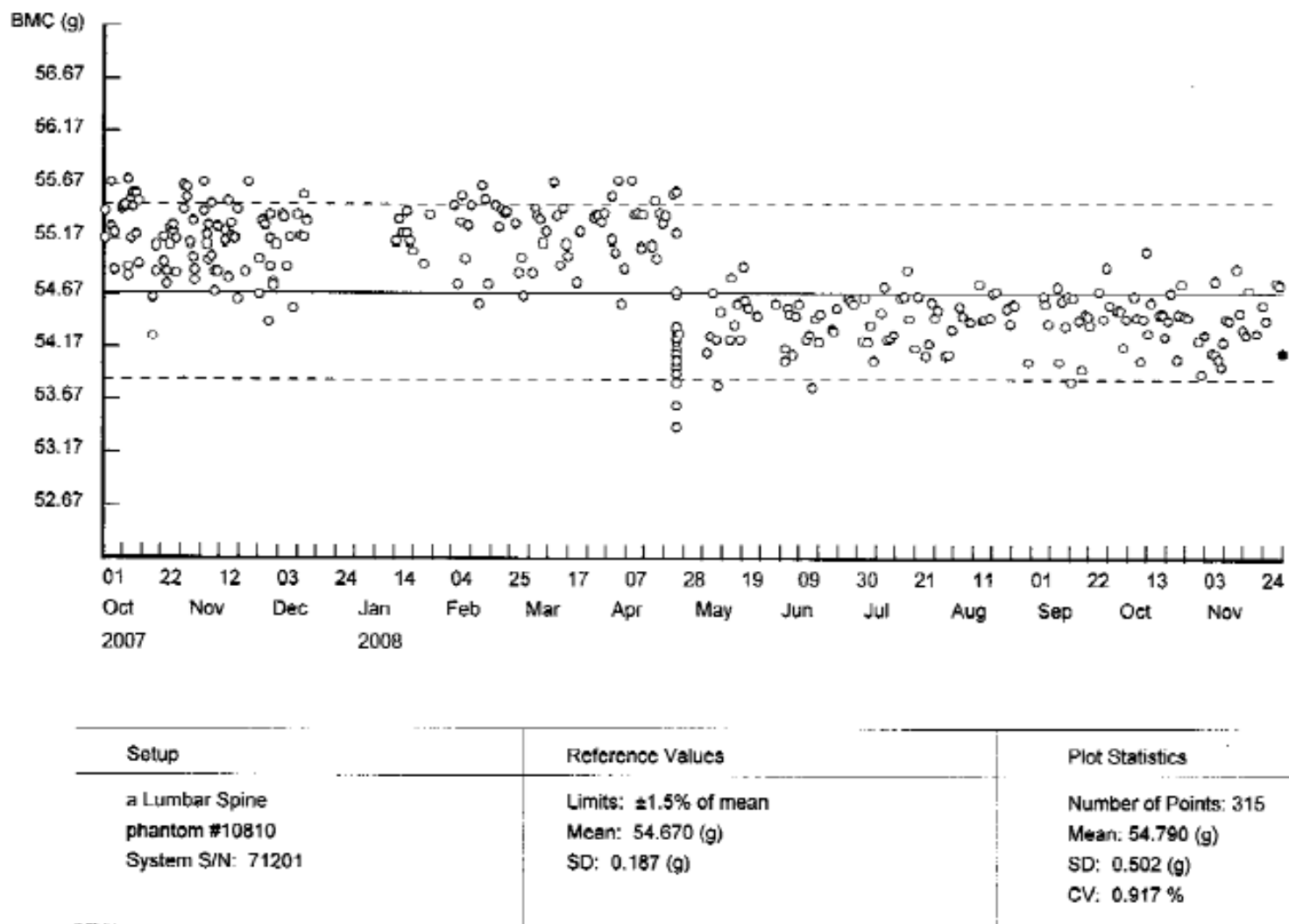
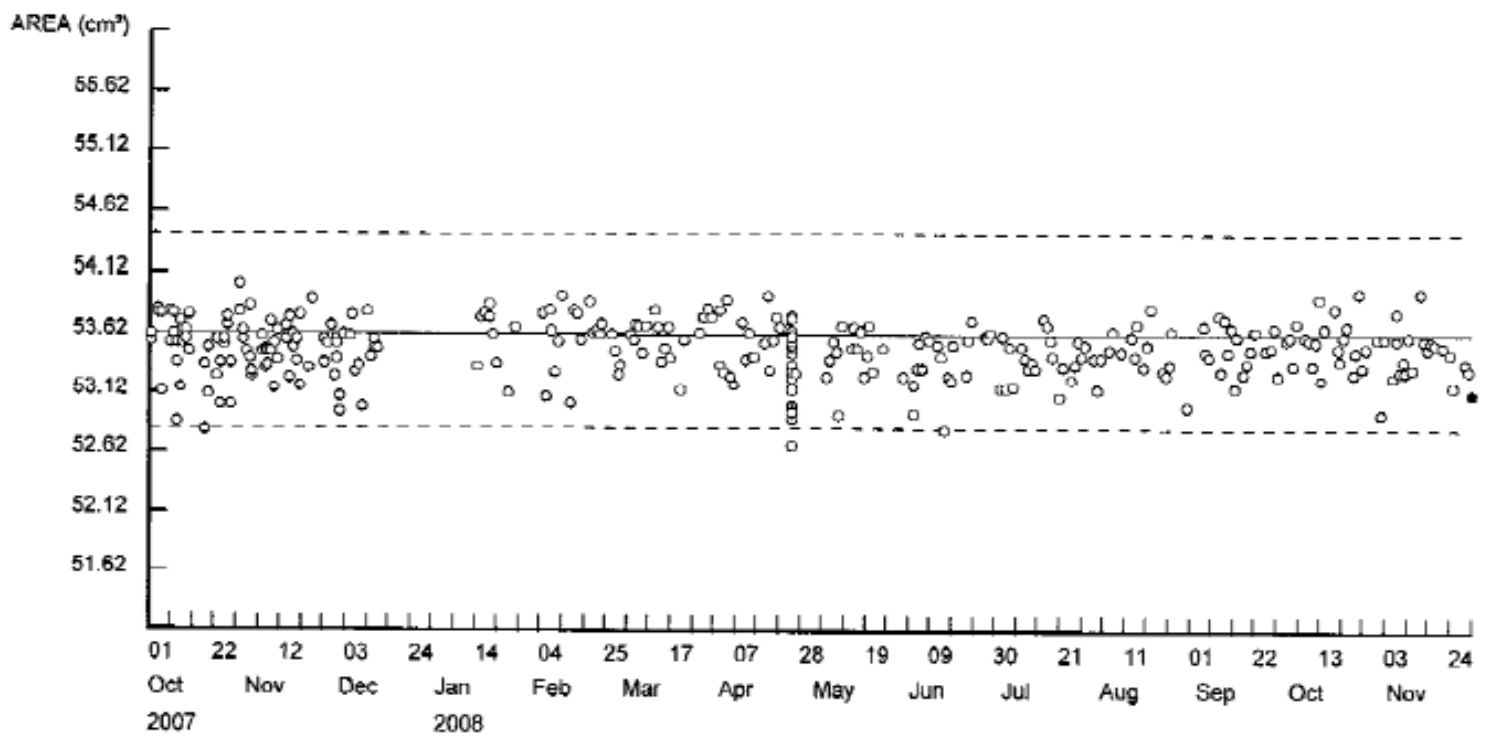


Figure 5-2: Phantom results for DXA machine form October 2007 till November 2008

BMC (g)




Setup	Reference Values	Plot Statistics
a Lumbar Spine phantom #10810 System S/N: 71201	Limits: $\pm 1.5\%$ of mean Mean: 53.615 (cm ²) SD: 0.007 (cm ²)	Number of Points: 315 Mean: 53.477 (cm ²) SD: 0.235 (cm ²) CV: 0.439 %

Figure 5-3: Phantom results for DXA machine form October 2007 till November 2008

Area (cm³)

6 Appendix 2: Ethics Clearance Certificate (M060423).

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	
Division of the Deputy Registrar (Research)	
HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL) R14/49 Meyers	
CLEARANCE CERTIFICATE	PROTOCOL NUMBER M060423
PROJECT	Does Nutritional Intervention Impact on the Outcomes of HIV-Infected Children Receiving ARV where Background mal...
INVESTIGATORS	Dr T Meyers
DEPARTMENT	Wits Paediatric Research Unit
DATE CONSIDERED	06.05.05
DECISION OF THE COMMITTEE*	APPROVED UNCONDITIONALLY
Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.	
DATE 06.07.06	CHAIR PERSON  (Professor M Vorster)
*Guidelines for written 'informed consent' attached where applicable	
cc: Supervisor: Dr T Meyers	
DECLARATION OF INVESTIGATOR(S)	
To be completed in duplicate and ONE COPY returned to the Secretary at Room 10005, 10th Floor, Senate House, University. I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. <u>I agree to a completion of a yearly progress report.</u>	
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES	

